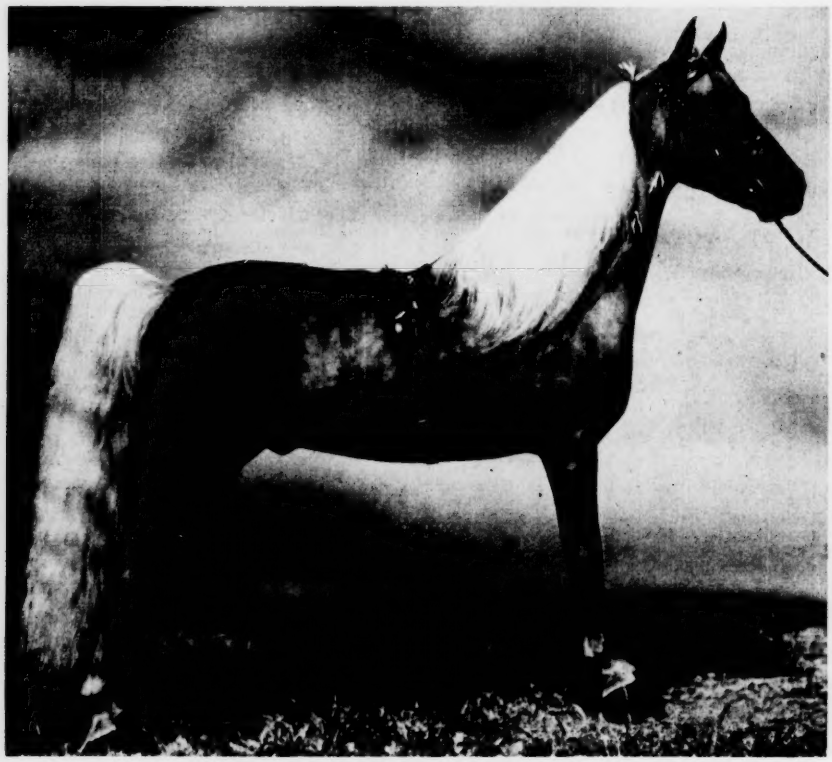
The Journal of HEREDITY



SILVER-DAPPLED SHETLAND PONIES

(SEE PAGE 139)

Sex Ratio in Mice Related to Blood pH

Race Crossing in Paradise

Photographs of Living Cells

VOLUME 44 JULY-AUGUST, 1953 NUMBER 4

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MEIOSIS I IN THE LUBBER GRASSHOPPER
Figure 1

All figures are of the lubber grasshopper, *Romalea dictyophorus*. *A*—Resting stage in which only minor coils are present. *B* to *I* are meiosis I. *B*—Prophase showing relational coiling of sister chromatids and major coils of the individual chromatids. Many gyres of relationa ➡

PHOTOGRAPHS OF LIVING CHROMOSOMES

L. R. Cleveland and A. M. Winchester*

UNLESS chromosomes may be seen clearly in the living condition, there is no way to end the controversy, which has existed for many years, regarding the effects of fixation and staining on their morphology. Electron microscopy does not help very much because material studied by this method must first be subjected to the drastic changes which result from dehydration. Ultraviolet light, polarized light, and darkfield illumination have helped somewhat in studying living chromosomes, but they are definitely inferior to certain types of phase contrast equipment.

Except for a limited use of the recent Leitz phase contrast microscope, the equipment used in this study was all specially prepared for us by the Bausch and Lomb Optical Company, to whom we are greatly indebted. The camera used was the regular B. and L. model L. The light source was 100 w. zirconarc and, for the most part, Isopan and Contrast Process Panchromatic films were used. These were developed in D 11 and DK 50.

Under the best possible conditions, one can always see with the microscope a good deal more than one can photograph. In most instances we have been able to see all the features of chromosomes that have been observed after fixation and staining and just as clearly. A few features, such as the chromatids of tetrads, are plainer.

In order to show in the photograph as much as possible of what one sees with the microscope by focusing, it is often advisable to flatten the material somewhat by exerting a small amount of pressure on it. This is a common cytological procedure that has been employed for many years, particularly in the study of chromosomes, and a considerable amount of criticism — most of which under the circumstances was justifiable — has been leveled at it. And the users of such a procedure, so long as

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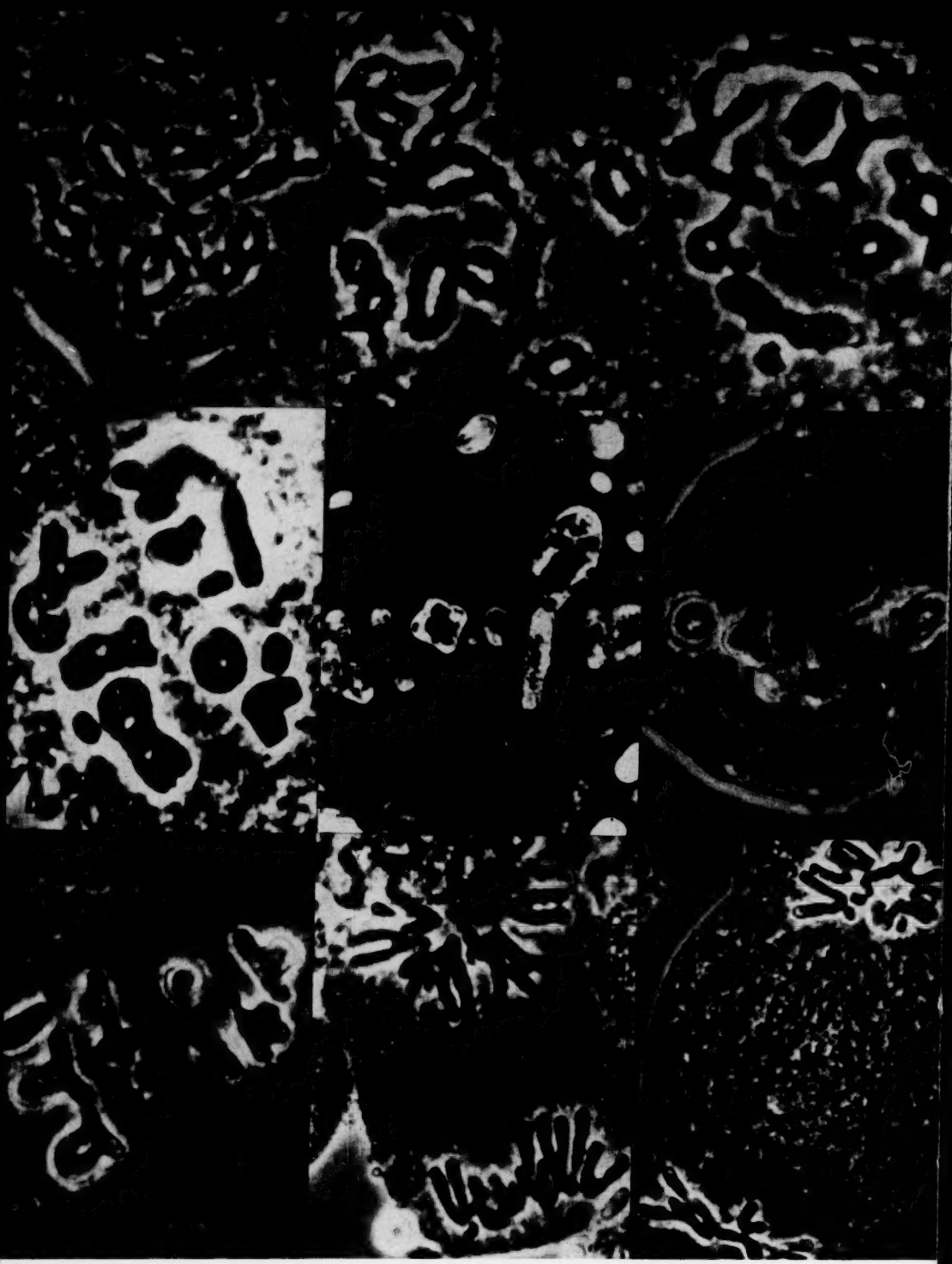
coiling are present, and because of this, doubleness of chromosomes (individual chromatids) may be seen clearly in only a few places along each chromosome. *C*—Later prophase. Only a few gyres of relational coiling in sister chromatids remain. This coiling has come out by unwinding. Note long distances where sister chromatids lie side by side (not coiled about each other). Major coils of the individual chromatids may be seen more clearly in the regions without relational coiling. The majors are still quite loose and irregular. *D*—Prophase pairing of chromatids has begun. Relational coiling and individual coiling of chromatids can be seen, but not as clearly as in earlier prophases. The gyres of major coils are much closer together, and hence less distinct. Chromatid pairing makes relational coiling less distinct. *E*—Considerably later prophase. Chiasmata have formed in several of the tetrads. They were produced as a result of alternate pairing of sister and non-sister homologous chromatids. This is shown best by the tetrad on right. The lower tetrad has formed a ring because in the center only chromatids are paired for a long distance; while the tetrad slightly above the center of the nucleus has not and will not form a ring because both sister and non-sister chromatids are paired except at the ends. In other words, this tetrad has chromosome pairing in its mid-region and chromatid pairing at its ends. *F*—A similar stage in which the chiasmata of the three tetrads at the lower border of the nucleus may be seen plainly. The longer one on the right has three chiasmata. *G*—Several of the tetrads of this nucleus have formed rings due to the fact that most of the pairing is between chromatids only. *H*—Note particularly the tetrad in the center which is forming two rings. At the left hand end of this tetrad, the ends of the four chromatids may be seen plainly. Tetrads of the three small chromosomes lie at the lower right hand border of the photograph. *I*—Note interlocking in tetrad to the left and slightly below center. An exchange in chromatid partners is fairly plain in the lower chiasma of the tetrad on the extreme right.



MEIOSIS I IN THE LUBBER GRASSHOPPER
Figure 2

All figures are of meiosis I in *Romalea dictyophorus*. *A*—Several of the tetrads are forming rings, but in this photograph the camera was focused on the haploid X-chromosome which lies slightly to the right of center and below two rings. The pairing of its sister chromatids is ➤

fixation and staining methods were employed, could not answer the criticism because they were not able to see the chromosomes clearly before compressing them. In other words, they had no way of determining whether pressure altered the chromosomes or not since they could not see them before exerting the pressure. With phase contrast methods, unless a preparation is unusually thick, one can see chromosomes clearly by focusing up and down before compressing them, and thus determine to what extent pressure alters them. Long focus objectives are excellent for such observations. It is amazing how much pressure most chromosomes will withstand without disruption. Of course, they become flatter, slightly longer, and straighter.

The salivary gland multiple chromosomes, however, are an exception. We have not been able to develop a method for flattening and untangling them without first treating them with dilute acetic acid. If compressed in insect Ringers, as were the grasshopper and other chromosomes for example, they disrupt. But they may be seen well enough before disentanglement and compression are attempted to be sure that the acetic acid treatment, which prevents their disruption, alters them very little.

All the chromosomes except those from the salivary glands of *Drosophila* were photographed in the living conditions from fresh preparations. Neither the negatives nor the photographs were retouched in any way whatever.

Material

In choosing organisms whose chromosomes were to be photographed an effort was made, in so far as availability of material permitted, first to select some of those that have been studied most by fixation and staining methods, and second to select as wide a variety of material as possible. It soon became evident that there was no point in photographing a large number of stages in the chromosome cycle of each organism, because those in all the organisms were too much alike in a given stage. We decided to use those of the Florida lubber grasshopper, *Romalea dictyophorus*, for a more detailed study mostly because the chromosomes of grasshoppers have been studied extensively. However, those of the spiderwort, *Tradescantia paludosa*, and the American newt, *Triturus viridescens*, are also well known and may be photographed readily in all stages. But those of the root tip of the onion, *Allium cepa*, for the most part, are not first-rate material for such purposes. Nor are those of the crayfish, *Cambarus bartoni*, in certain stages of their cycle, because of the large numbers present. However, some photographs were made of the chromosomes of all of these organisms, as well as those of the salivary glands of two species of *Drosophila*, *D. melanogaster* and *D. persimilis*. The chromosomes of many other cells were studied but not photographed.

Stages in the Meiotic Chromosome Cycle

Resting. In all stages of its cycle, a chromosome is the same length, although it appears much longer in some stages than in others. These apparent differences are brought

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plain (since it is haploid, there are no non-sister chromatids, and, of course, no chiasmata can be formed). *B*—Some of the tetrads in this figure are longer than those of previous figures and hence show earlier stages in the formation of chiasmata. *C*—Note particularly the tetrad slightly to right and above center which has chromosome pairing in the middle and only chromatid pairing for a considerable distance at each of the four ends. Compare this tetrad with the one below it which has formed a perfect ring and the one to the right which is forming a ring in the center. Thus, the loci of chiasmata—and hence the appearance of a tetrad—clearly depend on the manner in which sister-non-sister chromatids pair. *D*—Compressed metaphase. The width of the tetrads is exaggerated somewhat by the pressure. However, at this stage, they are considerably shorter, and broader than in Figures 1*E* and 2*C*. It is also more difficult to see chromatids and chiasmata in them. Achromatic figure was disrupted. Note the X-chromosome (upper right). It does not become so short and broad as the others. Its two chromatids vary considerably in the intimacy of their pairing. Here they are paired more closely than usual. *E*—Very much like the previous figure except light contrast. Major coils may be seen in the chromatids of the X-chromosome (lower right). These chromatids are not paired so closely as those of the X-chromosome in the previous figure. *F*—This is a metaphase-anaphase in which very little pressure has been applied to the cell so as not to disrupt the broad achromatic figure which extends across most of the cell and becomes narrow at each end as it approaches the centrioles which produced it. *G*—Anaphase that was compressed considerably, yet chromosomal orientation was not disrupted. *H*—Telophase. *I*—Later telophase.

MEIOSIS II IN THE LUBBER GRASSHOPPER
Figure 3

All figures are of *Romalea dictyophorus; B* to *I* are meiosis II. *A*—Telophase I after cytokinesis to show the central spindle portion of the achromatic figure still connecting the daughter cells. The central spindle, at this stage, fairly often is divided into two portions and is surrounded by mitochondria which give it a more compact appearance. *B*—Late prophase. Note slight pairing of chromosomes. In most instances the pairing is near ends. *C*—Similar ➳

about by uncoiling and recoiling.[1] During the so-called resting or least active stage, the chromosomes of most cells appear longer than at any other stage in their cycle because only minor coils are present in their chromonemata. In all cells where this is the case, the main difference between the chromosomes lies in the size and number of minor coils. In most cells, these coils are so fine, so numerous and there are so many superimposed layers of them that a surface view, which is the best one to photograph, gives a somewhat hazy image (Figure 1*A*).

In resting nuclei and through most of the prophase, nucleoli, which in most cases for sake of chromosomal clarity we have tried not to photograph, are present and may be seen clearly. One can also, in some instances, see clearly the stalk or stem that joins a nucleolus to a chromosome.

Prophase I. One can detect doubleness of chromosomes in the early prophase. Of course, the two chromatids, of which each chromosome is now composed, cannot be seen from one end of a chromosome to another, but there are places here and there along each chromosome where chromatids may be seen clearly (Figure 1*B* and 4*A*). At the same time, one can see that each chromatid is producing a new generation of major coils. As these chromatids become apparently shorter, their major coils become larger in diameter, closer together, and fewer in number (Figure 1*C*). At the same time the sister chromatids are losing their relational coiling (Figures 1*B* and *C*).

Pairing. However, before all the relational coiling of sister chromatids is lost pairing of meiosis I begins and prevents further loss of this type of coiling through the process of unwinding (Figure 1*D*).

It is not our purpose to consider in detail here the manner in which pairing begins and how chiasmata are produced, for this has been done in a forthcoming paper,[2] but it does seem desirable to point out that in the tetrads of the grasshopper, as well as those of other organisms, which were studied but were not photographed, the chromatids and the manner in which they pair may be seen clearly in living cells (Figures 1*E* to 2*C*). In certain tetrads, in early stages, there can be no question regarding the fact that for a considerable distance pairing occurs only between chromatids (Figures 1*E* to 2*C*). Also in some instances, one can see an exchange of partners in the paired chromatids. Chiasmata, of course, are very plain. The X-chromosome, which is haploid before meiosis I begins, forms no tetrads and of course no chiasmata (Figures 2*A*, *D* and *E*).

Metaphase—anaphase I. As the chromosomes take on a still shorter appearance and thus enter the so-called metaphase, the chiasmata and the individuality of the chromatids gradually become less distinct and finally, in some instances, become invisible (Figures 2*D* and 5*A*). However, in early anaphase, as the chromosomes begin to move apart in opposite directions, chromatids and chiasmata become clear again (Figures 2*F* and *G*). The decline in clarity of chromatids and chiasmata, of course, is due to the pairing of both sister and non-sister chromatids or, in other words, to chromosome pairing for a considerable distance along each tetrad. It might also be mentioned at this point, that a ring-shaped tetrad is produced when chromosome pairing is interspersed by a region where only chromatids are paired (Figures 2*B* and *C*). For example, the early tetrad in the center of Figure 1*H* will later form two rings, one being slightly larger than the other, while that at the bottom of Figure 2*A* will form only one large ring, and the one slightly above center in Figure 1*E* will not form a ring. Ring formation occurs more often in the salamander tetrads (Figure 4*B*) than in those of the grasshopper (Figures 1*E* to 2*C*).

No special effort was made to photograph the achromatic figure. If it happened to be in focus, or if it was not disrupted in flattening the chromosomes, which was the usual occurrence, it was photographed. It first appears shortly after pairing begins. At first, it consists in the grasshopper and salamander, where it was observed most, of two astral centers, each of which surrounds a ring-shaped centriole from which the astral rays radiate. When first observed, these centers lie a fair distance apart, usually on opposite sides of the group of tetrads. In a short time some of the astral rays from one center meet and join those from the other and thus form the small, early central spindle. This process of the astral rays from one centriole meeting and joining those from the other is repeated several times before the central spindle becomes large and the movement of chromosomes begins. In fact, the chromosomes do not begin to move until a large number of rather firm, but widely dispersed, central spindle connec-

to previous figure, except some homologues are clearly not paired at ends. In earlier prophases the homologues are not connected in any way. *D*—Early anaphase. Even though a fair amount of pressure was exerted on this cell and most of the achromatic figure was disrupted, one can see the poles faintly (the pressure pushed them some distance away from the chromosomes). *E*—Telophase. Note how long the chromosomes are. *F*—Later telophase. Chromosomes are shorter. *G*—Some of the chromosomes are greatly compressed. Note their major coils. *H*—Late telophase with the central spindle surrounded by mitochondria and extending across most of the elongated cell. *I*—Practically the same stage as the previous figure, but light contrast.

tions are made between the two centrioles (Figure 2*F*). The central spindle does not take on a compact formation—such as it does in the early stages of its formation in many flagellates and a few other forms—until mid-telophase, at which time it becomes more compact, as the cell elongates preparatory to cytoplasmic division (Figures 3*H* and *I*), and it persists for some time even after cytokinesis is complete. At this time, and sometimes earlier, the central spindle consists of two parallel portions (Figure 3*A*). This is seen more often at the end of meiosis I.

It is an easy matter at this time, as well as earlier, by pressing on the cover glass, to determine how very rigid the central spindle is. It certainly has sufficient strength to push the chromosomes apart as it increases in length (owing to growth processes), and that is exactly what observations on it in living cells indicate it does. It has an easier task in those cells where the nuclear membrane disappears in early stages of cell division than in those where the nuclear membrane persists until very late telophase when it is reorganized. In the first type of cells the nuclear membrane gets out of the way and does not have to be depressed before the central spindle can become central in position. Hence, there is no need for such a large compact structure as occurs in many flagellates where the central spindle must either depress or surround the nuclear membrane before it can function in chromosomal movement. Nor does it have the added burden, as it does in flagellates, of pushing the nuclear membrane in two in order to push the daughter chromosomes apart. Thus, there is clearly no need for such a large, strong, compact central spindle in metazoan cells as there is, for example, in the unusually large flagellate *Barbulanympha*.

Since the unique material for achromatic figure studies offered by *Barbulanympha* has been photographed and will appear in a forthcoming paper,[3] a detailed photographic account of this structure in such forms as the grasshopper, the salamander, and other metazoan forms appears unnecessary in the present paper.

Telophase I. In order to get most of the chromosomes in the same focal plane, one must exert sufficient pressure on them to disrupt the achromatic figure (compare Figures 2*H* and *I* of meiosis I where the chromosomes have flattened considerably wih 3*H* and *I* of meiosis II where they have not flattened very much). However, in the telophase after cytokinesis, a considerable amount of flattening (as well as other types of mistreatment) is necessary before disruption of the central spindle begins (Figure 3*A*).

Prophase II. We have made no effort to determine when the chromatids of I become completely separated. Each puts in its own new generation of major coils (Figure 4*C*). This begins in early prophase and is completed before pairing of homologues starts. The homologues do not come together and pair until fairly late prophase. The degree of pairing varies considerably at the same stage in different nuclei, several of which have been photographed, although only two are shown (Figures 3*B* and *C*). Pairing begins more often near the ends; however, it may begin at any point. The homologues are scarcely ever paired for their entire length. The chromosomes in this prophase II pairing, particularly in the early stages (Figure 3*B*), present an appearance not greatly unlike that seen in late telophase I (Figure 2*I*).

Metaphase—telophase II. The chromosomes of metaphase II appear considerably longer (Figure 3*D*) than those of I. This is also true as they go to the poles in anaphase (Figure 3*E*), but by late telophase (Figures 3*H* and *I*) they are not much longer than in the same stage of I. They retain their major coils throughout this period, although the gyres fit so close together that these coils cannot be seen clearly except when the chromosomes are considerably elongated by pressure which forces the gyres apart (Figure 3*G*).

Stages in the Mitotic Chromosome Cycle

Prophase relational coiling of sister chromatids and the major coils of individual chromatids may be seen in the tissues of many animals and plants. Some, however, show these two types of coils a good deal more clearly than others (Figures 5*B*, *E* and *F*). At metaphase (Figure 5*C*) the chromatids appear longer than at the same stage in meiosis. The same is also usually true at telophase (Figure 5*D*).

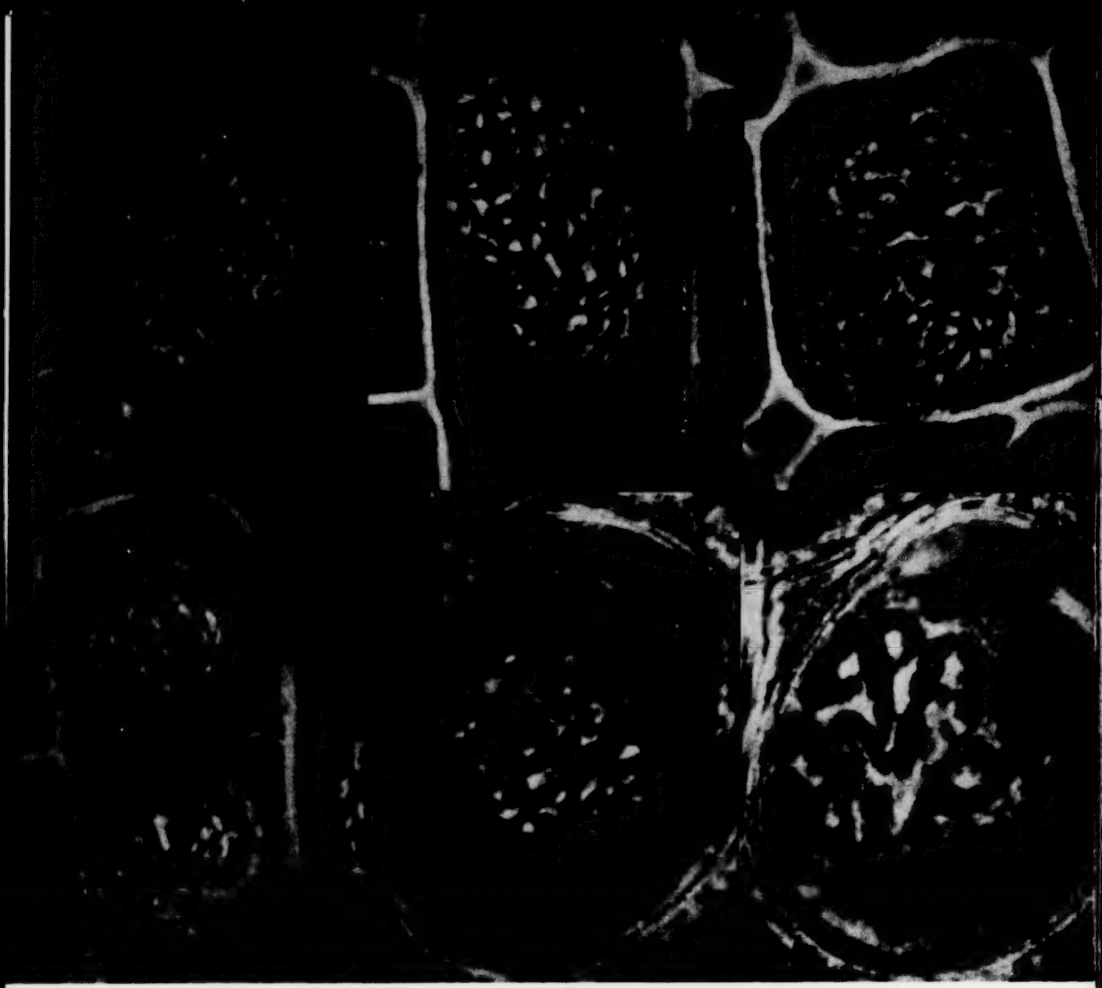
Salivary Gland Chromosomes

The so-called bands of these multiple or polytene chromosomes may be seen quite clearly with certain types of phase contrast equip-

THE AMERICAN NEWT AND *DROSOPHILA*

Figure 4

A to *D* are of the American Newt *Triturus viridescens*. *A*—Prophase I. A surface view of the nucleus to show relational and individual major coiling of chromatids. *B*—Late prophase I. Most tetrads, owing to the manner in which the chromatids paired, have formed rings. *C*—Prophase II. Note several stages in the developmnet of a new generation of major coils. *D*—Telophase I. *E* to *H* are of polytene chromosomes from the salivary glands of *Drosophila*. *E*—Portions of several chromosomes of *D. melanogaster*. *F*—Most of one chromosome of *D. persimilis*. *G*—An inversion in *D. persimilis*. *H*—Light contrast photograph of portions of chromosomes of *D. persimilis*.



THE CRAYFISH, ALLIUM AND SPIDER-WORT
Figure 5

A—Metaphase I of the crayfish, *Cambarus bartoni*. *B*—Prophase mitosis in onion root tip. Relational coiling of sister chromatids may be seen in places. *C*—Mitotic metaphase of onion root tip. *D*—Late telophase of onion root tip. *E*— Fairly early prophase of haploid mitosis in pollen grain of *Tradescantia paludosa*. *F*—Later prophase of same. Note relational coiling of sister chromatids and the individual major coils of chromatids.

ment (Figures 4*E* to *H*). Their width and thickness varies as much, if not more, than in fixed and stained material, and one can see quite clearly that they are neither bands nor discs, but rings. The so-called double bands are sometimes not double at all. A single, somewhat flattened ring may appear double when its top and bottom lie in almost the same focal level.

In no instance were any of the negatives or prints retouched. All figures except 2*E*, 3*I* and 4*H* are dark contrast.

Magnification for all Figures is approximately 3,000 ×.

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Nutritive Value of Teosinte

Little is known of the nutritive value of teosinte (*Euchlcana mexicana*), although it is the closest known relative of corn (*Zea mays*) . . . .

The nitrogen values for teosinte are considerably higher than those for corn. . . . Of even greater significance is the correspondingly higher methionine content of teosinte, more than twice that of corn. Methionine is now recognized to be the limiting amino acid in the predominately vegetable diets of most underdeveloped areas of the world. The peoples of these areas must obtain increased amounts of methionine in their diets, but for basic agricultural, economic and cultural reasons, this problem cannot be solved entirely by an increase in the production of animal protein. Therefore, vegetable products, which have a high supplementation value in the mixed diets of these areas and which can be produced readily, are urgently needed. From the data presented teasinte should be further studied as a potential source of vegetable protein of relatively high methionine content.

The amounts of tryptophane and lysine /100g in teosinte do not appear to differ significantly from those in corn. On the basis of suggested minimum daily requirements for these amino acids, the quantity of lysine is probably sufficient even with the increased proportion of methionine in teosinte. Tryptophane, however, must certainly be a limiting amino acid in teosinte protein. . . .

Teosinte corn hybrids can be readily obtained, but five such crosses (F_1) analyzed as part of the present study did not differ significantly from corn in their chemical composition although the plants and ears were intermediate in structure and appearance. However, their progeny (F_2) should show marked variation in nutrient composition. The yield of some plants grown from wild seed in Antigua reached 500 g, although the range of variation was great. This would indicate that the yield per acre of selected seed on good ground is potentially high.

Ground teosinte can be mixed with wheat or corn flours or used alone to make biscuits, tortillas, and other products. In view of the widespread efforts to improve the world supply of protein for human consumption, the relatively high proportions of total protein and methionine in teosinte are potentially important. The direct use of teosinte for food in mixed vegetable diets, as well as the possible improvement of the protein content of corn by hybridization with teosinte and subsequent selection for both yield and nutritive value, should be seriously investigated.

Irving E. Melhus, Francisco Aguirre and Nevin S. Scrimshaw
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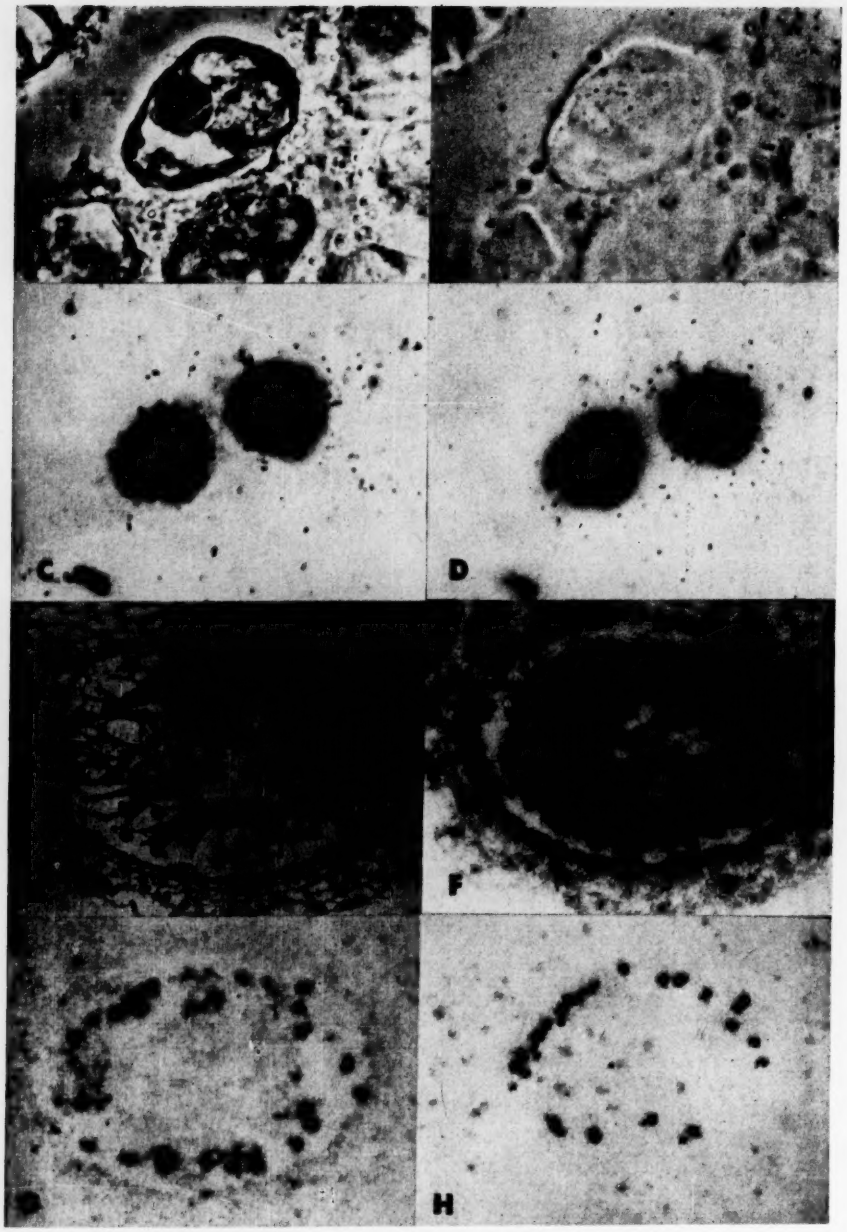

AUTORADIOGRAPHS OF *TRADESCANTIA* AND LILY
Figure 6

A—Phase photograph of unstained pollen grain of *Tradescantia* (7μ section); *B*—Same section photographed with bright field, showing localization of silver grains over the generative nucleus (×1,200); *C*—Nucleus of pollen mother cell at pachytene and tapetal cell on the right smeared after the Feulgen reaction; *D*—Same nuclei at a higher focal level showing a concentration of silver grains over the tapetal nucleus which has incorporated P^{32} in the DNA (×1,450). *E*—Cross-section of a locule of the anther of *Lilium longiflorum* stained with safranin and fast green; *F*—Autoradiograph of a similar unstained section after incorporation of S^{35} and extraction with alcohol-acetic acid fixative and cold TCA; *G*—Similar section showing autoradiograph after digestion in pepsin; *H*—Autoradiograph after digestion of the section in pepsin and pectinase (×120).

THE AUTORADIOGRAPH — A TOOL FOR CYTOGENETICISTS

J. Herbert Taylor and Shirley H. Taylor*

AUTORADIOGRAPHS may now be prepared with sufficient resolution to follow the distribution of radioisotopes within individual cells.[4] This opens the way for tracer experiments in following the biochemical activities of individual nuclei or even single chromosomes. The two most interesting substances from the genetic viewpoint, nucleic acids and proteins, can be readily labeled with phosphorus 32 and sulfur 35, respectively. In some cases selective labeling is possible and in others selective extraction utilizing specific enzymes or acid hydrolysis will produce cells with only one component labeled. Howard and Pelc[5, 6] have used these methods to follow nucleoprotein synthesis during the mitotic cycle in cells of bean roots. Taylor[15] followed nucleic acid synthesis during meiosis and pollen development in *Tradescantia* and lily. A combination of the autoradiographic technique with cytochemical methods being developed for measurements at the cellular level[1, 12, 13] promises to advance rapidly our knowledge of gene reproduction and function.

Besides certain technical problems of autoradiograph preparation and the differential extraction of labeled compounds, the significance of incorporation of isotopes remains to be solved. Although there are many biochemical data on the relations of growth, synthesis and incorporation of isotopes in nucleic acids and proteins, most of the data cannot be interpreted in terms of events in individual cells. Presumptive evidence indicates that incorporation of P^{32} into desoxyribonucleic acid (DNA) is limited to periods of synthesis, but similar correlations for isotope incorporation in ribonucleic acid (RNA) and proteins require clarification. High resolution autoradiographs, that can detect intracellular distribution of a radioisotope may help in this problem, in addition to serving as a tool for certain cytogenetic problems.

Phosphorus 32 as a Tracer for DNA Synthesis

Phosphorus 32 (available as H_3PO_4) can be applied to cells in anthers of *Tradescantia* or lily by placing freshly cut inflorescences in mineral solutions containing 0.02 mg. of KH_2PO_4 and 20 μC of P^{32} per ml. of solution. The P^{32} reaches the pollen mother cells in detectable amounts in three to five hours when the stems are cut three to four inches long. If the cells are at stages during which incorporation occurs, eight to 20 hours later the cells have incorporated sufficient P^{32} in the nucleic acids to produce an autoradiograph. The tissues are fixed in alcohol-acetic acid (3:1) and the cells are affixed to microslides by squashing or as tissue sections (5-7μ thick). Non-nucleic acid phosphorus must be removed by appropriate extraction. The fixative removes most of the acid soluble phosphates. Following fixation and after the cells are on slides they are extracted for five minutes in hot ether-alcohol (1:3) to remove remaining phospholipids. After passing through the alcohol series, the cells are stained by the Feulgen method. The 10 minute hydrolysis in 1N HCl removes the RNA phosphorus and any acid soluble phosphorus that may remain. Most of the remaining phosphorus is in the desoxyribose nucleic acid. Some protein phosphorus is present, particularly at late pollen stages, but this can be distinguished by comparison with autoradiographs of cells extracted with hot trichloroacetic acid (TCA) at 90° C. for 30 minutes or after digestion in desoxyribonuclease.

After the appropriate extraction procedure, the slides are coated in the darkroom with autoradiographic stripping film (available from Kodak, Ltd., London, as glass plates coated with 10 microns of gelatin and 5 microns of emulsion). Rectangular pieces about 1 by 1½ inches may be cut, stripped off the glass and floated emulsion side down on water. The film is then lifted up on the slide in such a way that the emulsion is in intimate

*Columbia University. This investigation is supported by a grant from the Atomic Energy Commission, Contract AT (30-1)—1304.

contact with the cells. The preparation is dried thoroughly and stored in the dark in a moisture-proof box for two weeks. The film is developed and fixed while still attached to the slide. The slide is passed through an alcohol series and mounted as a permanent preparation.

Examination of these slides usually shows high concentration of silver grains over some nuclei and few over the others (Figure 6*A-D*). In the developing anthers where numerous cells of sporogenous tissue and tapetum show synchronous development, the uptake of phosphorus in the DNA of nuclei can be correlated with cytological stages. In sporogenous tissue incorporation in DNA occurs during a relatively short period (eight to 12 hours) just prior to meiotic prophase in lily and during preleptotene or early leptotene in *Tradescantia*. No further uptake occurs during the two divisions of meiosis. The next uptake in DNA is during the period 24 to 36 hours preceding the prophase of the microspore division in lily and 12 to 24 hours before this prophase in *Tradescantia*. Another uptake occurs in the generative nucleus during mid-interphase of the maturing pollen grain.[15] Later experiments have shown that a large fraction of this phosphorus can be removed by hydrolysis of the tissue sections in five percent TCA for 30 minutes at 90° C. Digestion of comparable sections in desoxyribonuclease likewise removes most of the same fraction of phosphorus. With a low background grain count and an autoradiograph that is weak enough for counting individual silver grains, quantitative data can be obtained. Quantitative data for analysis of the effectiveness of the acid and enzyme hydrolysis in removing phosphorus from acetic-alcohol fixed nuclei will be published elsewhere. However, it may be stated that the phosphorus remaining after the Feulgen hydrolysis is almost exclusively DNAP at the stages mentioned. At other stages detectable amounts of phosphorus do not become incorporated into DNA. The published data of Swift[14] derived from photometric measurements of DNA in pollen mother cells and developing pollen of *Tradescantia* indicate an increase corresponding to each of the periods during which DNA incorporates phosphorus.[15] If this correlation is a regular one, a new method for detecting chromosome reproduction is available. It means that the DNA molecule has a very stable backbone of phosphoric acid linkages and that appreciable incorporation of phosphorus occurs only when additional molecules are synthesized. Further experiments to check the correlation of synthesis and incorporation of phosphorus are under way.

During the stages of meiotic prophase the tapetal cells show a highly synchronous behavior which is correlated with the stages of the adjacent pollen mother cells. Tapetal nuclei fail to incorporate phosphorus into DNA synchronously with the pollen mother cells at the beginning of meiotic prophase. Incorporation in tapetal nuclei occurs during interphase, when the pollen mother cells are at zygotene. There follows shortly a synchronized mitotic division of all tapetal nuclei. After early leptotene the pollen mother cells neither incorporate phosphorus in DNA nor acquire any labeled DNA from tapetal cells, contrary to morphological evidence presented by Cooper.[2]

Incorporation of Sulfur 35 Into Cellular Proteins

Sulfur 35 (available as H_2SO_4) was administered by placing the cut inflorescences in a mineral solution containing 0.036 mg. of $MgSO_4$; 7 H_2O and 10 μC of S^{35} per ml. Thereafter autoradiographs were prepared in a manner similar to that used after P^{32} incorporation.

Sulfate is rapidly incorporated into the sulfur-containing amino acids which the plants would be expected to synthesize. Autoradiographs, prepared after fixation of cells in alcohol-acetic acid and extraction with cold TCA after mounting on the slides, show incorporation of sulfur into the proteins of the anther cells at all stages of development. However, the amount of sulfur incorporated varies by a factor of 20 to 1 at different stages. For example, in the microspore of *Tradescantia* very little sulfur is incorporated by the cell while the nucleus is dividing, but at later stages in the maturing pollen at least 20 times as much becomes incorporated during a period of similar length.

Although it has been shown that cystine may become incorporated into proteins by forming disulfide linkages with free sulfhydryl groups of proteins even in boiled homogenates,[10] it is not clear how important such incorporation may be in these experiments. The rate of incorporation of sulfur into proteins in these cells reflects the rate at which amino acids are being synthesized and this in turn might be expected to reflect the rate of protein synthesis in the cell. The differences of incorporation in adjacent layers of cells in the same anther are often very striking. The tapetal cells in the lily anther incorporate 10 to 20 times as much sulfur as the adjacent pollen mother cells during late pachytene (Figure 6*E* and *F*). This probably reflects differences in rate of protein synthesis, certainly of metabolic activity, of the two types of cells. At late premeiotic interphase and early leptotene the situation is reversed. Incorporation is very slow in the tapetal cells but very rapid in the adjacent pollen mother cells.

An attempt has been made to determine the time of incorporation of sulfur into the chromosomal proteins with a view to finding by

this means the time of their duplication. One of the complicating factors is the incorporation of sulfur into the cytoplasmic proteins and into the chromosomes simultaneously. The scatter of beta particles from cytoplasm interferes with the interpretation of the chromosomal autoradiograph. Another site of binding of sulfate is in cell walls, particularly the heavy exine of the lily pollen grain. Most of the cytoplasmic sulfur could be removed by digestion of the section of tissue in pepsin (Figure 6*G*). The crystallized pepsin (Worthington Biochemical Co.) was used in a procedure similar to that of Kaufmann *et al.*[7] (2 mg./ml. of solution at pH 1.6 for two hours at 37° C., preceded by immersion of the slides in 0.2 molar lanthanum acetate for five minutes). After removal from the enzyme solution the slides were rinsed briefly in cold TCA, left five minutes in lanthanum acetate and then stored two or three hours in 30 percent alcohol before the film was applied. The remaining extranuclear sulfur could be removed from the cells of the young anthers by digestion in partially purified pectinase (Figure 6*H*) for two hours at room temperature. However, this was not sufficient to remove all of the sulfur bound in the thick exine of the pollen grains. Since pectinase purified by the method of Lineweaver *et al.*[8] would be expected to have little activity on anything except the pectic substances, the sulfate may be bound to the galacturonic acid residues of these compounds. Although it is difficult to keep the tissues on the slides after such drastic treatment, it is often possible to get nearly complete sections by mounting tissue directly on clean slides without an adhesive (Figure 6*H*).

It is apparent that pepsin is very efficient in digesting cytoplasmic proteins, while it leaves the chromosomes nearly intact in these cells. Some shrinkage of the nucleus occurs and perhaps some of the sulfur is lost from the nucleus. However, leptotene and pachytene chromosomes of lily remain structurally identifiable. Nucleoli remain but appear as ghosts with the phase microscope, whereas in undigested sections they are quite dense. The undigested chromosomal proteins contain most of the sulfur of the nucleus, but their nature remains in doubt. From the experiments of Mazia *et al.*[9] in which pepsin did not digest fibers of nucleohistone, the undigested proteins might be expected to be primarily of the histone type. On the other hand, experiments of Daly *et al.*[3] have shown that histones are digested by pepsin and contain relatively little sulfur. Perhaps these undigested sulfur-containing proteins correspond to Mirsky's "residual proteins."[11] In our experiments incorporation of sulfur into nuclear proteins is most rapid at the periods when DNA synthesis occurs. This may indicate that the chromosomal proteins are synthesized at the same time as the DNA, but incorporation also occurs at all times when the cell is metabolically active, that is, when the cytoplasm incorporates appreciable sulfur in its proteins. Therefore, the time of synthesis of the protein of the chromosomal nucleoproteins cannot be determined with certainty until that difficulty is resolved.

A further word of caution should be added concerning the interpretation of the autoradiographs after digestion with pepsin. The possibility of diffusion and exchange of sulfur-containing amino acids and peptides during digestion should be considered. For example, if by some means sulfur-containing peptides or amino acids removed from the cytoplasm should be bound in the nucleus during digestion, the retention of sulfur by the nucleus could in part be an artifact. Control sections of similar lily anthers which did not contain sulfur 35 were placed on the same slides with the radioactive tissues during digestion. The nuclei of these control sections did not pick up detectable amounts of the isotope. Although these sections are subjected to lower concentrations of the products of digestion than nuclei in the labeled sections, this experiment indicates that exchange is probably not a significant factor in contributing sulfur-containing proteins to the nucleus.

Two observations on the sulfur incorporation should be emphasized. (1) The chromosomes have a sulfur-containing protein which is not digested by pepsin as used in these experiments. (2) There is a striking reduction of incorporation of sulfur into the proteins of cells during stages of division when the chromosomes are condensed. This would indicate that chromosomes play an immediate, essential role in the synthesis of all cellular proteins, a role that is quickly reflected when they are not in a metabolic state. It should be noted at the same time, however, that incorporation may be very slow at certain stages when the nucleus is in interphase. An example is the tapetal nuclei at the stage when the pollen mother cells are in early leptotene.

Incorporation of phosphorus into DNA appears to be a good means of detecting its synthesis. The same may prove to be true for RNA. Until the different mechanisms of incorporation of cystine-sulfur into proteins can be distinguished, incorporation of sulfur administered as sulfate can at best only reflect the rate of amino acid synthesis. This, of course, may be a good measure of the rate of protein synthesis in plants, where sulfate is utilized in synthesis of amino acids.

Summary

A technique is described whereby the distribution of a radioisotope can be detected in different parts of a single cell. By the application of this technique the time of incorporation of phosphorus 32 and sulfur 35 has been followed in cells of anthers of lily and *Tradescantia*. DNA incorporates phosphorus only at the beginning of meiotic prophase, in the

interphase a few hours before prophase of the microspore division, and again about mid-interphase in the pollen grain. The chromosomes show the fastest incorporation of sulfur at these same times, but they also incorporate sulfur at other times. Therefore, synthesis of the DNA portion of the chromosomal nucleoproteins can be readily detected, but the continuous incorporation of protein sulfur makes the timing of protein synthesis less definite. Digestion of sulfur-labeled cells with pepsin shows that pepsin digests the cytoplasmic proteins and leaves the sulfur-containing proteins in the nucleus. With further improvements in differential extraction of cellular components, autoradiographs will be valuable for the cytogeneticist to detect chromosome duplication and presumably gene reproduction. In addition the movement of large molecules within the cell can be followed.

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GENETICS IN THE U.S.S.R.

MORE than thirty years ago the authors determined, by cytological and genetic analysis, that the cultivated wheat varieties originated by natural hybridization of basic species accompanied by chromosome doubling to produce new allopolyploid species. The more primitive species of wheat and allied genera have seven pairs of chromosomes. Many thousands of years ago spontaneous crosses between some of these basic species, accompanied by chromosome doubling, gave rise to the *Emmer* series of wheat species with 14 pairs of chromosomes. These *Emmer* wheats produced hybrids with other basic species which, when accompanied by chromosome doubling, gave rise to the *Vulgares* wheat species with 21 pairs of chromosomes. These 42-chromosome *Vulgares* species include our bread wheats, while the 28-chromosome *Emmer* series include the *Durum* or Macaroni wheats.

Cytologists and geneticists throughout the world have verified and accepted these conclusions. The final proof was provided when McFadden and Sears in the United States and Kihara and his associates in Japan resynthesized the 42-chromosome *Vulgares* species by crossing 28-chromosome *Emmer* wheat species with the 14-chromosome *Aegilops squarrosa* and doubling the chromosome number. These synthetic 42-chromosome species are similar to the 42-chromosome *Vulgares* species, and when crossed with them they produce fertile hybrids.

The new generation of Soviet "scientists" now claim that it is possible to transform the 28-chromosome *Emmer* wheats directly into 42-chromosome bread wheats simply by changing the environment—time of planting, soil conditions, or growing the plants in a warmer or cooler region. Even more fantastic claims

(Continued on page 158)

ASSOCIATION OF BLOOD-pH WITH SEX RATIO IN MICE

J. A. WEIR*

THERE has been ample opportunity to discover anomalous sex ratios in mammals since sex at birth, or at some later stage in development, is recorded by all breeders or investigators who keep systematic records. Data which survive the test in the struggle for statistical significance will reveal real differences but will not shed light on the causes for variations in sex ratio. To get at the real causes some measure of the nature of genotypic control over sex ratio is necessary. Discovery in the present investigation that changes in sex ratio arose as concomitants of selection for both high and low blood-pH has afforded an opportunity to examine the influence of a single, gene-controlled character on the basic sex ratio. Association of an excess of males with high blood-pH and excess of females with low blood-pH suggests that genetic constitution of the mother may set up chemical conditions which in turn may act on the sperm of the father to favor or handicap either the *X* or *Y* type, making for differential sex ratios in the resulting litters. In that this theoretical interpretation is subject to confirmation through work in progress, this paper must be regarded as a preliminary report.

Are the Differences in Sex Ratio Real?

The manner of formation of the high blood-pH line (designated pHH) and low blood-pH line (designated pHL) from an outbred strain of mice (designated T) will be discussed in a later section. In our laboratory the sex of each individual is recorded at weaning time, when the mice are 30 days of age. Data for the high and low blood-pH lines are presented in Table I. Since the excess of males in the pHH-line and excess of females in the pHL-line could be due to differential survival of the sexes before the time for weaning, it is necessary to have some record of sex ratio at birth. This has been obtained by selecting from the data of Table I only those litters in which the number born exactly equals the number weaned. The ratio so obtained does not differ from the 30-day sex ratio indicating that mortality from birth to weaning affected both sexes equally. Moreover, if the observation of MacDowell and Lord[14, 15] that fetal regression in mice is non-differential with respect to sex, applies to our material, the secondary sex ratio should closely approximate the primary sex ratio.

The probability that the pHH- and pHL-lines of mice belong to one sex ratio population is remote indeed (Chi-square = 11.70). It can be seen from Table II that differences in percent males between pHH and pHL, which range from 0.6 percent in generation 4 to 32.4 percent in generation 5, are positive for all generations. Large sampling errors are of course likely in the generations which include few litters. Significance of attainment of the overall sex ratio difference of 10 percent, in the first generation of selection for blood-pH, will be referred to later.

Comparison With Other Laboratory Strains

Table III allows comparison of the pH-strains with the T-strain, from which the pH-lines were derived, and with LCH-(leucocyte count high) and LCL-(leucocyte count low) lines which were selected in the same manner as the pH-lines and from the same initial stock. The only difference is that the criterion

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of selection was blood-pH in the one case and total leucocyte count in the other. The amount of inbreeding has been approximately the same in pH- and leucocyte-lines.

The T-mice are arranged in Table III to correspond in time with the generations of selection for leucocyte count. For the first four generations the leucocyte-lines exactly parallel the pH-lines. After that the generations did not correspond in time. Examination of environmental influences is therefore valid only for the first four generations.

The stability of sex ratio in the T-strain is apparent. The only significant deviation was in the "sixth" generation and that was the first generation raised in Kansas. Upon transfer to the University of Kansas from the University of Saskatchewan there was a complete change in environment including a new diet so that a change in sex ratio might be expected. If the "sixth" generation data are omitted the T-sex ratio becomes 50.3 percent. Whether or not the drop in proportion of males was due to environment, it was not permanent. Since a decline in propotion of males had already occurred in the leucocyte lines of mice before transfer from Canada the reason for the "sixth" generation drop in the T-strain remains obscure. It could be an accident of sampling.

Using the T-strain as a base, the difference in sex ratio between pHH- and pHL-lines is striking in that the deviations are in two directions from the 50:50 ratio. This suggests that both an increase in blood-pH and a decrease in blood-pH are effective in altering the basic sex ratio. Sex ratio in the lines selected on a basis of leucocyte count is more variable than in the outbred T-line but in general the high and low leucocyte count lines tend to vary together.

Is the Association of Sex Ratio and Blood-pH Accidental?

There is nothing remarkable about strain differences in sex ratio and there is no reason to expect an excess of males as a general phenomenon. It is well known that in sheep, for example, an excess of females is the rule. In mice an equality of sexes appears to be the most usual situation[8]; this is the case in

TABLE I. Sex Ratio at 30 Days of Age of High Blood-pH and Low Blood-pH Lines of Mice

	pHH Mice					pHL Mice				
Generation	Number of Litters	♀♀	♂♂	Total	% ♂♂	Number of Litters	♀♀	♂♂	Total	% ♂♂
1	23	100	102	202	50.5	19	100	70	170	41.2
2	22	82	101	183	55.2	20	80	75	155	48.4
3	24	85	104	189	55.0	22	91	82	173	47.4
4	20	72	87	159	54.7	25	99	87	186	46.7
5	6	18	25	43	58.1	6	11	8	19	42.1
6	2	1	5	6	83.3	3	4	4	8	50.0
7	1	4	4	8	50.0	8	32	31	63	49.2
8	7	18	27	45	60.0	10	30	17	47	36.2
9	11	31	51	82	62.2	9	32	15	47	31.9
10	6	13	22	35	62.8	5	13	8	21	38.1
	122	424	528	952	55.46	127	492	397	889	44.66

55 Litters (427 T mice) from which pHH and pHL first generation mice were derived had sex ratio of 48.01 ± 2.70% ♂♂.

TABLE II. Sex Ratio at Birth of High Blood-pH and Low Blood-pH Lines of Mice

	pHH Mice					pHL Mice				
Generation	Number of Litters	♀♀	♂♂	Total	% ♂♂	Number of Litters	♀♀	♂♂	Total	% ♂♂
1	13	58	65	123	52.8	11	56	42	98	42.8
2	11	45	55	100	55.0	6	23	24	47	51.1
3	12	49	55	104	52.9	13	56	51	107	47.7
4	10	40	40	80	50.0	11	44	43	87	49.4
5	5	12	23	35	65.7	2	6	3	9	33.3
6	2	1	5	6	83.3	1	1	2	3	66.7
7						5	22	22	44	50.0
8	6	16	23	39	59.0	8	24	15	39	38.5
9	10	29	49	78	62.8	6	20	12	32	37.5
10	5	13	20	33	60.6	5	13	8	21	38.1
	74	263	335	598	56.02	68	265	222	487	45.58

38 Litters (321 T mice) from which pHH and pHL first generation mice were derived had sex ratio of 47.98 ± 3.28% ♂♂.

our outbred T- and inbred LCH- and LCL-lines. It is also true for the combined population of pHH and pHL mice. Inbreeding will fix alleles which influence sex ratio if such alleles are present in an initial outbred population. Continued inbreeding in the LCH- and LCL-lines of mice still lead to significant strain differences in sex ratio although that now seems unlikely. It would not have been at all surprising, however, if consistent differences had shown up as a result of inbreeding. The possibility of chance fixation of genes for sex ratio in the selected pH-lines must therefore be examined. This can be done by briefly reviewing the manner of formation of the various lines of breeding. A more detailed discussion of blood pH will be presented elsewhere.

The T-strain derived from MacArthur's[12, 13] stock, is being maintained by outbreeding. Means and variances for blood-pH, leucocyte count, resistance to mouse typhoid and proportions of segregating colors (black, black and tan, leaden, brown, albino) have remained stable. The percent males from 55 litters containing 427 mice, in generation 0, was 48.01 ± 2.70. In 38 litters (321 mice) in which no deaths occurred between birth and weaning, the percent males was 47.98 ± 3.28. Two hundred and eighty T-mice of generation 0, chosen at random, were tested for blood-pH. The mean pH value was 7.458 ± .0044. Using blood-pH as the sole criterion of selection, seven males and 23 females were used to establish the high line and six males and 19 females to establish the low line. Inbreeding coefficients for the progeny were 2.27 for the pHH and 1.97 for the pHL indicating very little consanguinity at this stage.

The results of three generations of selection for blood-pH are shown in Table IV. The first generation means of 7.466 ± .0048 for the high line and 7.420 ± .0051 for the low differ by .046, which is significant beyond the 0.01 level. Two additional generations of selection resulted in no significant changes in the means although there was reduction in variability (heritability calculations yielded 23.1 percent for generation 1, 25.9 percent for generation 2 and 27.5 percent for generation 3). It appears that the number of segregating genes responsible for individual pH differences in the T-strain must be small, possibly no more than two. Otherwise there should have been continuous progress by selection. In the third generation, in which the inbreeding coefficient for the pHH strain was 20.8 and for the pHL 20.3, the data have been arranged according to the mating system used to produce them (Table V). Inbreeding did not lead to any more rapid fixation of genes for pH than did outbreeding. Blood-pH and resistance to mouse typhoid have remained stable, both being quite different in the two strains.[25]

The data in Tables I and II reveal that the changes in sex ratio followed exactly the changes in blood-pH. Assortative mating for blood-pH immediately separated the mice into "high-male" and "high-female" lines. The inference that the one may be responsible for the other is inescapable. Genetic linkages could be responsible but this seems less probable than a direct association since the sex ratio must have a physiological basis of some sort and pH is known to profoundly affect many physiological processes.

Although in the last analysis the heterogametic parent is normally responsible for the sex of any one offspring, he cannot be held wholly accountable for the statistical consequences of many matings. It may be that the higher proportion of males in the high blood-pH line and higher proportion of females in the low blood-pH line are functions of the dam's genotype. However, the solution to this problem must await the results of reciprocal crosses and double matings with color markers.

Sex Ratios in Man and *Drosophila*

No discussion of sex ratios in animals would be complete without mention of *Homo sapiens* and *Drosophila melanogaster*. In general, analyses of the voluminous data for humans have stressed the influence of general environ-

TABLE III. Sex Ratio at 30 Days of Random Bred (T), Leucocyte Count High (LCH) and Leucocyte Count Low (LCL) Strains of Mice

	T Mice				LCH Mice				LCL Mice			
Generation*	Litters	♂♂	Total	%♂♂	Litters	♂♂	Total	%♂♂	Litters	♂♂	Total	%♂♂
0	55	205	427	48.0								
1	22	93	179	52.0	13	50	96	52.1	15	57	111	51.3
2	21	98	196	50.0	13	39	86	45.3	14	48	95	45.2
3	11	44	82	53.7	16	60	117	51.3	16	48	118	40.7
4	16	70	148	47.3	19	71	158	44.9	20	69	147	46.9
5	15	65	123	52.8	20	43	105	41.0	16	46	115	40.0
6	14	47	113	41.6	8	14	33	42.0	17	35	87	40.2
7	14	67	134	50.0	12	57	103	55.3	12	49	82	59.8
8					17	74	145	51.0	8	30	43	69.8
9	16	66	125	52.8	15	50	100	50.0	17	70	132	53.0
10	18	83	159	52.2	13	63	111	56.8	20	79	165	47.9
11	17	86	170	50.6	14	65	116	56.0	6	19	42	45.2
	219	924	1856	49.78	160	585	1169	50.04	161	545	1137	47.93

*"Generation" refers specifically only to LCH and LCL mice. T mice are grouped by approximate correspondence of time of birth with that of LCH and LCL.

mental factors. The argument is that anything which increases or diminishes the chances for prenatal mortality will affect the sex ratio since abortions and stillbirths are believed to occur more frequently when male fetuses are involved. Although the theory is adequate to account for certain known facts, for example, the correlation between socio-economic class and sex ratio, its scientific validity has by no means been established. On the contrary, microscopic examination of the gonads of 5,787 embryos in the Carnegie Collection, ranging in size from 20-149 mm., revealed no significant variation in the proposition of males at any stage of development.[22] The actual ratio for abortions from the third to the seventh month of gestation (51.9 ± 0.7 percent males) was found to be about the same as for viable infants. Tietze's statement that official statistics may be grossly in error seems well founded since the Carnegie Collection apparently represents a random sample of fetal deaths. Despite the fact that postnatal deaths are more frequent in boys than in girls it cannot be stated with any degree of confidence that causes for variations in sex ratio at birth are ascribable to events of an obscure nature which occur only some time after fertilization. There is reliable evidence to the contrary for both mouse and man.

The problem of isolating specific genetic influences on sex ratio in humans is no less difficult than the approach through environment. Although the possibility of complete genetic control is indicated by the nearly exclusively male pedigree of Harris[9] and exclusively female pedigree of Lienhart and Vermelin,[11] we must look to *Drosophila* for an evaluation of the role of "sex ratio" alleles. Since initial discovery of the "sex chromosome" in animals, the theory of sex determination has advanced by successive steps so that one is no longer expected to fit sex ratio data into a Mendelian straight jacket. Quite apart from the well known effect of sex linked recessive lethals, there is an ever growing number of cases in *Drosophila* in which specific genes have been shown to have large effects on sex phenotypes and sex ratios.[4, 7]

Most pertinent to the present discussion is the discovery by Gowen[5, 6] of a sex factor in *Drosophilia* which leads to sex ratio variations from family to family even within a single line. The effect of this factor, which is found in at least two regions of chromosome II, is to push the progeny sex ratio toward the males. Breeding tests have ruled out elimination of sex chromosomes of the female or presence of a gamete lethal in the male and results of selection for high sex ratio indicate that the secondary inheritance is effective in modifying the effects of the sex factor. The proportion of the sexes became stationary at about 85 males to 15 females. Although the manner in which the male ratio factor operates has not been cleared up there is evidence that the missing females may be lost in egg or larval stages. The complexity of this thoroughly studied case in *Drosophila* indicates that a simple solution to the problem of variations in sex ratio in mice is unlikely.

TABLE IV. Blood-pH of Random Bred (T) Mice and Lines Selected for High and Low Blood-pH

Mouse Strain and Generation	Number of Mice	Blood pH Mean	Blood pH S. E.	Difference Between Means per Generation
T	280	7.458	.0044	
pHH-S1	180	7.466	.0048	
opHL-S1	165	7.420	.0051	.046*
pHH-S2	170	7.466	.0048	
pHL-S2	149	7.414	.0052	.052*
pHH-S3	183	7.476	.0041	
pHL-S3	169	7.426	.0040	.050*
T (Age similar to S2)	72	7.444	.0072	

* Significant beyond the 0.01 level.

The "Acid-Alkali" Problem

The present study will recall to readers of the JOURNAL OF HEREDITY the controversial acid-alkali sex control experiments of the thirties[1, 23] *et seq.* which purported alternately to prove or disprove that pH of the female reproductive tract at the time of conception may significantly alter the sex ratio. Unfortunately, quite apart from the popular interest in all matters pertaining to sex, or to the determination thereof, attempts to "confirm" the claims of the Unterberger school were of limited scope. As originally posed and publicized the problem presented a challenge to geneticists of a sort reminiscent of reappearances of Lamarkism in various guises. It is not surprising therefore that competent investigators, stung by an invasion of their field by amateurs, should approach the problem with limited objectives and extreme caution. Great efforts were made to duplicate exactly Warren's technique[23] for the most part although Cole *et al.*[2] developed a method to alter directly pH of the semen and at the same time permit a higher degree of standardization. Results of independent investigations reported in the JOURNAL OF HEREDITY (December 1940) and additional data by Quisenberry[19] and McPhee and Eaton[16] are summarized in Table VI.

Although the results of four independent experiments are inconclusive and conflicting they by no means completely dispose of the hypothesis that pH of the female tract may

TABLE V. Comparison of Systems of Mating Used in S-3 Generation of Blood-pH Mice

Mouse Strain	Mating System	Number of Individuals Tested	Mean Blood pH
pHH	Full sibs	101	7.475
pHH	Half sibs	26	7.471
pHH	Outcross	56	7.479
pHL	Full sibs	91	7.432
pHL	Half sibs	15	7.414
pHL	Outcross	63	7.420

influence sex ratio. Moreover, Roberts' data,[21] which show increasing effects with increasing concentration of douche, suggest a direct effect of pH *per se*. Cole *et al.*[2] obtained completely negative results, whereas Quisenberry and Chandiramani[20] obtained positive results with rabbits in the preliminary experiments only. Results of extensive experiments of McPhee and Eaton[16] were negative.

Of more interest in relation to the present study are the observations that cyclical changes in pH normally do occur in the female genital tract in humans.[10] Albeit the postulated significance of pH in relation to sex ratio may be wholly speculative, dismissal of evidence through recourse to theoretical physiology is not justified. For example, on a basis of logic, conception should never occur during menstruation as it sometimes does in humans, nor post-partum as it regularly does in mice.

Lamar *et al.*[10] found a positive correlation between pH of the cervical mucus in women and its penetrability by spermatozoa. The periods of high pH, which occur during the menstrual flow and at mid cycle, are the ones in which the mucus is most penetrable. On the other hand, between menstruation and mid cycle and between mid cycle and the subsequent menses, the cervical secretion is generally acid and penetrability is at a low ebb. The authors also state (p. 238) : "Experience shows that pH is not the sole factor."

Although changes in pH in the female tract have been established, the possible relation of these changes to sex ratio must, as yet, be regarded as wholly speculative. It is possible that in the high blood-pH and low blood-pH strains of mice differences of some magnitude exist in pH of cervical mucus. As pointed out in the writer's[24] reference to Menkin's data[17,18] even small differences in pH of blood, which is highly buffered, may be associated with larger differences at other sites. Moreover, the differences may be within the alkaline range entirely. It may be argued that *X*-bearing spermatozoa are slightly better able than *Y*-bearing spermatozoa to penetrate cervical mucus at unfavorable times and that the reverse is true under favorable conditions. Should this be true, an excess of females should be associated with unfavorable (i.e. relatively low pH) conditions.

Most of the innumerable theories of sex determination have been shown to be unsound in many or all of their essential features and those which do rely on accepted principles are frequently not susceptible to either proof or disproof. One theory, which is very old despite recurrent flurries in the press, is that sex of offspring is related to time of coitus during the estrus cycle. For a review of the subject the reader is referred to Crewe's paper.[3] Although the weight of evidence seems to be against the theory it could be that causes for conflicting evidence are bound up with a cyclical phenomenon. The superposition of experimental interference upon a naturally occurring pH cycle, the two working sometimes in harmony, sometimes in disharmony, could lead to just the sort of picture that has been encountered.

Admittedly these speculations go far beyond the evidence. It cannot be claimed that the present investigation sheds any real light on the problem of sex determination. Rather it is the acid-alkali concept which provides at least the framework of a working hypothesis to explain our findings in mice.

It is unlikely that the association of blood-pH and sex ratio in mice is fortuitous.

Summary

Selection for variations in blood-pH was successful, in one generation, in fractionating an outbred line of mice into a high pH line (blood-pH 7.466 ± .0048) and a low pH line (pH 7.420 ± .0051). Concomitant deviations in sex ratio, from the normal ratios in an outbred line, were at once observed. The first generation of high blood-pH mice, at birth, consisted of 52.8 percent males, the low blood-pH group of 42.8 percent males. The significant differences in both blood-pH and sex ratio have remained stable through nine subsequent generations of inbreeding. Contrasted to this the outbred control line has not devi-

TABLE VI. Summary of Acid-Alkali Experiments

Animal	$NaHCO_3$ % Concentration	$NaHCO_3$ Total Young	$NaHCO_3$ % ♂♂	Lactic Acid % Concentration	Lactic Acid Total Young	Lactic Acid % ♂♂	Investigator
Rat	3	283	55.5	2	274	40.5	
Rat	5	543	67.4	3	467	35.5	Roberts[21]
Rat	3–5	891	50.7	1–3–5	855	52.3 ± 1.7	Cole *et al.*[2]
Rabbit	2	170	47.6 ± 3.8	2	116	50.9 ± 4.6	
Rat Strain 1	3	269	50.6 ± 8.2	2	185	47.6 ± 6.8	Quisenberry
Rat Strain 2	3	118	50.8 ± 5.4	2	172	51.2 ± 6.6	and
Rabbit	3	47	44.7 ± 3.4	2	88	36.4 ± 4.5	Chandiramani[20]
Rabbit	5	49	65.3 ± 3.3				
Rabbit	5	93	54.5	3	121	51.6	Quisenberry[19]
Rabbit	5	855	50.2	2–3	710	50.1	McPhee and Eaton[16]

ated significantly from equality of sexes and sex ratio in lines selected on a basis of total leucocyte count, though somewhat variable, is also expressed by equality of sexes. A direct physiological relationship between blood-pH and sex ratio of offspring is postulated.

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SILVER DAPPLE, A UNIQUE COLOR VARIETY AMONG SHETLAND PONIES

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A FAVORITE color variety, with breeders and owners of Shetland ponies in the United States, is called *silver dapple* or *dappled chestnut*. This so-called chestnut variety is of quite different genetic constitution from the chestnut of horses and ponies in general. It is really not chestnut at all, which in horses is a basically brown pigmentation, but is black diluted to a dark cream color by action of a dominant modifier, which we shall call gene *S* (for silver dapple).

The idea that "dappled chestnut" Shetland ponies are really modified or diluted black in color has for some time been held by the junior author in common with several other experienced breeders of ponies. The first clear expression of this idea which has come to our notice was made by Ralph Armstrong in the January 1950 issue of the *American Shetland Pony Journal*, in which he pointed out the analogy and certain differences between the diluting action of gene *D* on brown pigmentation in the genesis of a Palomino horse and the action of the hypothetical modifying gene which we call *S*, on black pigmentation in the dappled chestnut Shetland pony.

He noted this difference between the two cases. "The silver dapple dilution factor reduces black to a color very similar to its counterpart in chestnuts, and in both cases the mane and tail are white or silver.

The Palomino dilution factor produces a white or nearly white mane and tail in chestnuts with a cream or gold body color, but in blacks it produces a dark mane and tail with dun body color.

What usually pass for Palominos in registered Shetlands are cream colored silver dapples and light golden sorrels with very light mane and tail."

Salisbury[4] made a tabulation of the recorded colors of 14,700 Shetland ponies, grouped in relation to the colors of their parents. Of these colts 797 were recorded as chestnut and 692 as sorrel. How many of those recorded as chestnut were genuine chestnut and how many were modified black, it is impossible now to state. But it is safe to assume that chestnut and sorrel ponies recorded as such prior to 1886 were genuine, since the modifying gene *S* was unknown prior to 1886, when it apparently originated by mutation.

Salisbury naturally assumed, as did the senior author until recently, that the white-maned chestnuts and sorrels found among Shetland ponies are of the same genetic constitution as white-maned chestnuts and sorrels among horses, which have been demonstrated to be basically brown pigmented. But white-maned dappled chestnut and sorrel Shetland ponies are basically black pigmented, as Armstrong has stated.

Genes of Shetland Ponies

Four principal genes are involved in the production of the dappled chestnut color variety of Shetland ponies. These genes may be designated by the symbols *A*, *B*, *E* and *S*.

The first three occur in horses and ponies generally; the fourth is peculiar to Shetland ponies, in which it seems to have originated by mutation about 1886.

1. *B* is the symbol for black, a gene active in the production of black pigment in the skin and hair of all mammals. When its action is not restricted or modified by other genes, black pigment is produced throughout the hairy coat of the entire body. A recessive allele (alternative form) of *B* is designated *b*. It is found in many species of domesticated mammals, and it acts exactly like *B* except that the end product is brown pigment instead of black.

Of the three remaining genes, *A* and *E* act to restrict or limit the distribution of black-brown pigment in the coat, and *S* modifies its quality.

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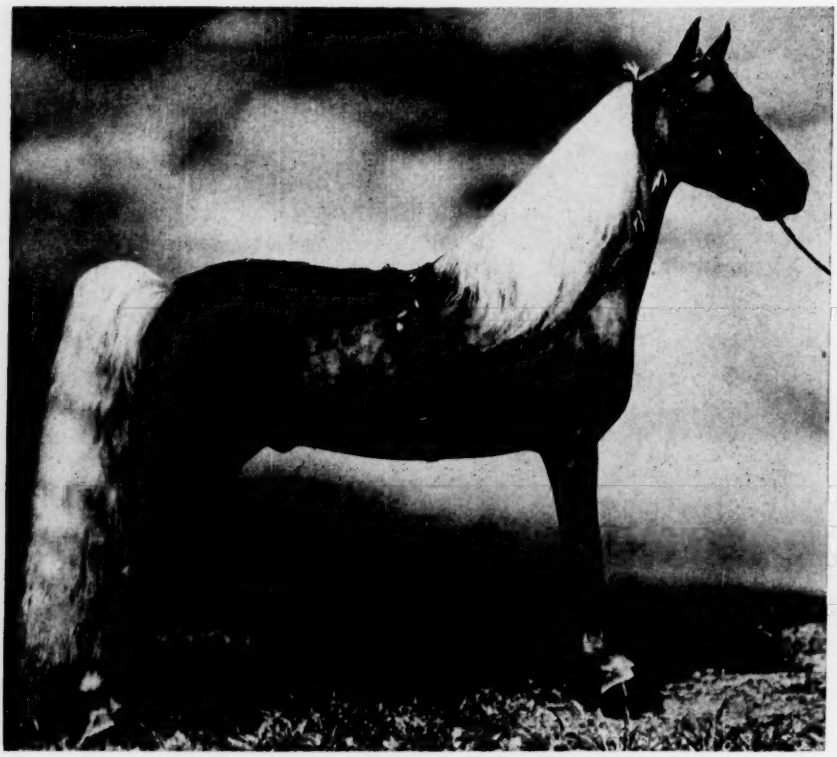


"SILVER WHITE'S SURPRISE"
Figure 7

A typical dappled chestnut pony, "Silver White's Surprise," whose probable genotype is *A–B–E^{D}–S–*. (Courtesy of the owner, John W. Norman, Winters, Texas.)

Whenever in the coat of a mammal the distribution of black-brown pigment is restricted by gene action, the black or brown pigment is replaced by yellow-red pigment, which has a different chemical composition. We may now give attention to two restricting genes found in horses as well as in many other mammals.

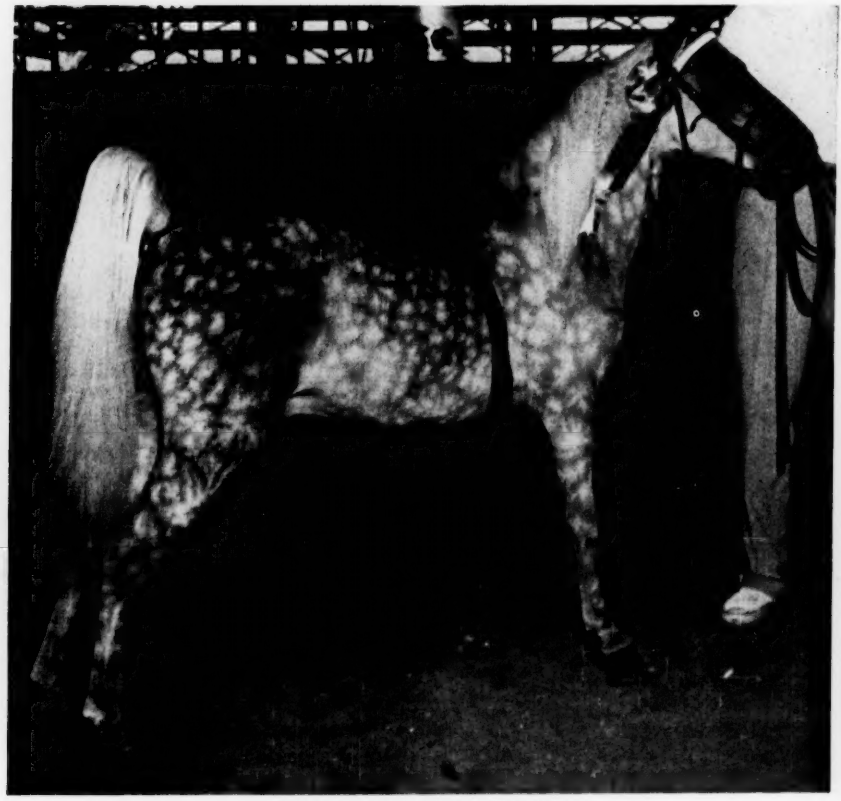
2. *A* is the gene for wild coat-pattern, which is the agouti of rodents. In horses the primitive coat pattern is still found in the wild Prejvalski horse of Mongolia, and persists in a form more or less modified in domestic bays, "browns", and chestnuts. We shall for convenience speak of it as the bay pattern.

A in horses restricts black-brown pigment for the most part to peripheral areas, such as the head, lower part of legs, spinal stripe including especially the long hairs of mane and tail. The central part of the body becomes largely yellow-red pigmented. The bay pattern is also characterized by lighter spots on muzzle, flanks, belly, and inner sides of the legs.

A recessive allele of *A* is designated *a*. It involves total absence of the bay pattern. In the absence of other restricting genes, the *aaB–* genotype is uniform black known as *recessive black* because in a cross with a genetically pure bay, the progeny are all bay.

The genotype *A–B–* is bay in appearance, *A–bb* is chestnut, *aaB–* is recessive black, and *aabb* liver chestnut, the brown counterpart of recessive black.

3. The gene *E* (symbol for *extension*) permits black-brown pigment to be distributed uniformly throughout the coat in the absence of *A*. Its recessive allele *e*, when homozygous *restricts* the distribution of black-brown pigment in a way similar to the action of *A*, but not in an elaborate concealing pattern as does



"SILVER WHITE'S CADET"
Figure 8

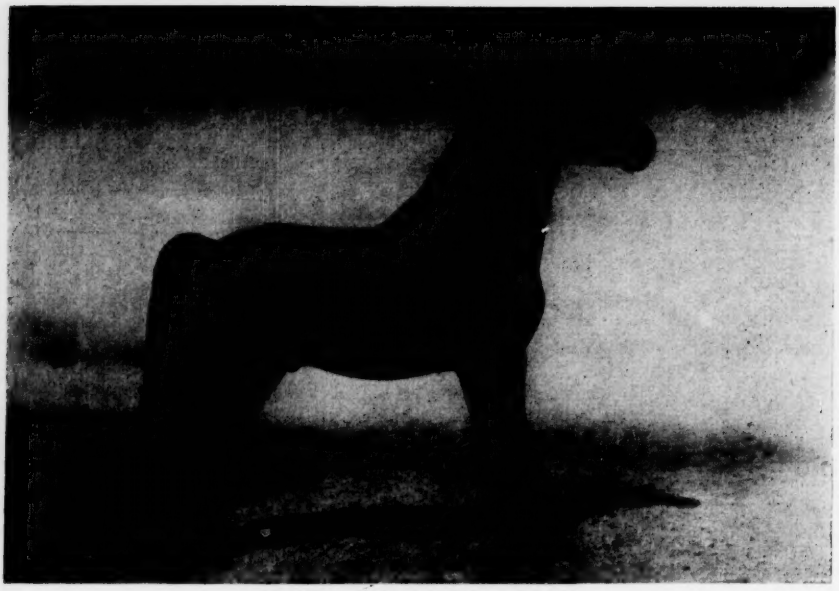
A dappled chestnut pony, "Silver White's Cadet," showing unusually well the dappled body, with a "mask" on the face. Compare the mane and tail, less clear in this case than in Figure 7. The probable genotypic difference, *A–* in Figure 7, is replaced by *aa* in this figure. (Courtesy of the owner, Crescent Pony Farm, Arlington, Texas.)

A. It allows black-brown pigment to be developed chiefly in peripheral areas, thus reinforcing the action of *A*. Together the two restricting agencies, *A* and *ee*, render the central body areas of the coat almost completely devoid of black-brown pigment, wholly occupied by yellow-red pigment; at the same time mane, tail, and legs are black or brown. Examples are seen in red bay horses (*A–B–ee*) and sorrel horses (*A–bbee*).

The restricting influence of *A* without that of *ee* would be seen in the genotype *A–B–E–* which (we suppose) would approach the Prejvalski horse in color pattern, having considerable black in the body but with distinctive "light points." It would be an unimproved, inferior type of bay.

The restricting influence of *ee* without *A* would be seen in the genotype *aaB–ee*. This would be an imperfectly developed black. Black would predominate peripherally but not centrally. It might perhaps be popularly called "brown," or "Brown-black."

A dominant allele of *E* is called E^D. It increases the production and extension of black pigment throughout the coat beyond that resulting from the action of *E*. The production of black pigment is so great in the genotype *A–B–*E^D*–* that the bay pattern scarcely shows at all. Indeed it is probably completely hidden when E^D is homozygous, for such is the case with the agouti pattern in rabbits where the E^D allele was first discovered. The genotype *B–*E^D*–* is known as *dominant black*. When to



"SHOREACRES BLACK LARIGO"
Figure 9

A dominant black pony, "Shoreacres Black Larigo," 25687, whose probable genotype is A–B–E^D–. (Courtesy of the owner, Dr. Wayne A. Munn, Janesville, Wisconsin.)

this combination A is added, as by a cross with agouti in rabbits, or bay in horses, the wild pattern scarcely shows, and will be completely hidden when E^D is homozygous, as in the genotype A–B–E^DE^D.

Dominant black was probably introduced into the silver dapple strain by Prince of Wales, the intense black stallion which sired the foundation sire Chestnut. Naturally Chestnut did not show the bay pattern, though he may have possessed its gene concealed by E^D. (Compare Figure 9.)

4. The gene S, found only in Shetland ponies, modifies black of even the most intense character to a shade resembling dark cream. The first pony known to have shown this color (doubtless a mutation) was the mare Trot 31 (in the records of the American Shetland Pony Club), born in 1886, the color being described as "fawn."

Her owner, Mr. C. E. Bunn, in a letter to the junior author written in 1947, says this about Trot.

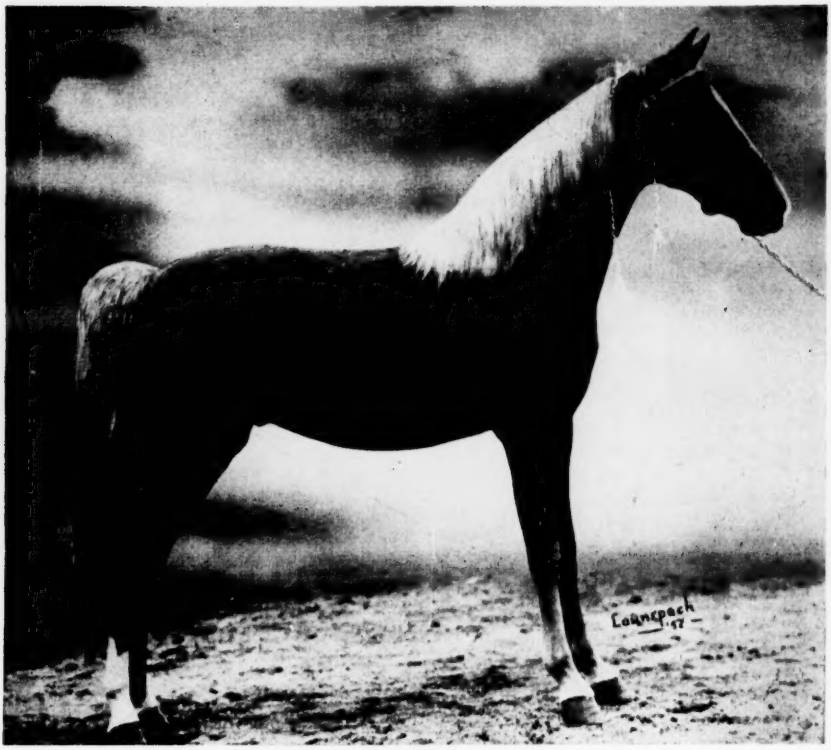
"She was the only pony of this color in America and was also a beautiful color. She was in foal when I bought her and produced [a mare colt] Keithsburg. This mare had the flax mane and tail but was not the dappled chestnut like her dam. [She would now probably be classed as sorrel.] However I bred her to Prince of Wales [an intense black stallion, probably B–E^D–] and the result was Chestnut who took the color of his grand-dam Trot [dappled diluted black with white mane and tail.]

When I showed Chestnut he was such a sensation that I was deluged with calls for this class of ponies. I sold many yearling stud ponies and many mares in foal to Chestnut and in a few years this color was often seen. Many were very high class and some, of course, were not so good, as they were produced from all sorts of mares, good and bad.

However, every one of the ponies you now see of this color was the produce or descended from Chestnut. His grand dam Trot was the only mare ever known to have that color of all the Shetlands ever bred or known. [Students of genetics call such abrupt origins mutations.]

This was not a red sorrel with flax mane and tail, as there were a number of these, but a peculiar color more like a dark cream, with the dapples showing in the hide. In the winter, with the long coats, you did not see the dapples and the color looked more like a dirty white, not pretty. But when the pony shed in the spring and the real color showed up, they were beautiful."

Mutant modifiers of black, similar in actior



"RED CHESTNUT KING"
Figure 10

A red sorrel pony, "Red Chestnut King," 30038, whose probable genotype is *A–B–eeS–*. (Courtesy of the owner, Dr. W. E. Verploeg, Pella, Iowa.)

to that of *S* in Shetland ponies, have long been known in laboratory rodents (rats and mice), the only apparent difference being that in rodents the modifiers are recessive in heredity, whereas the *S* of Shetland ponies is dominant.

In rats a mutation, also named fawn, when homozygous, modifies black pigment to a shade resembling chocolate, but not identical with it either in genotype or in appearance.

In both rats and mice, a mutation called pink-eye (*p*) renders black pigment so pale that its presence in the retina of the eye can be established only by examination with the microscope. The coat of pink-eyed rats is bright yellow in the presence of the *A* gene, which permits development of true yellow pigment in the subterminal bands of the hair. But when *A* is absent, as in the non-agouti genotype *aaB–pp*, modified black pigment occurs throughout the length of the hairs, which are a dull cream color, showing no true yellow pigment.

These facts are mentioned to show that genes modifying black pigment, so that it resembles cream or even brown in appearance, are well known in mammals other than horses, which fact makes the existence of *S* as such a modifier in Shetland ponies a reasonable hypothesis.

In Shetland pony records, prior to the birth of Trot, many are recorded as bay, black, chestnut or sorrel in color. These were probably equivalents of the corresponding colored sorts in horses, and their genotypes as follows:

Bay *A–B–E–*
Black *aa B–E–*(or *A–B–E*^{D}*–*)
Chestnut *A–*(or *aa*) *bbE–*
Sorrel *A–*(or *aa*) *bbee*

If the stallion Chestnut was mated with "all sorts of mares," as stated by Mr. Bunn, then it is probable that nearly all conceivable combinations were produced of the gene *S*, with genes *A* or *a*, *B* or *b*, and *E*^{D}, *E*, or *e*.

Combinations which did not include *E*^{D}

GRAY-WHITE PONIES
Figure 11

Two gray-white ponies (of a string of six) bred by the owner, (man with hat). Their probable genotype is *aaB–E^D–G–S–*. (Courtesy of the owner, Fred Wilmot, Richards, Missouri.)

would fall short of the sensational contrast effects of dappled body and white mane and tail found in Chestnut. If so, *B–E–S–* combinations would be only medium dark silver dapples, and *bbS–* combinations would be very light perhaps to such a degree as to merit prompt discarding. No such individuals have been positively identified.

The genotype most likely to be selected for prepetuation would be *A–* (or *aa*) *B–E^D–S–*, and these would be recorded as "dappled chestnuts," which they would be in appearance but not in reality, since they, like their ancestor Chestnut, would really be modified dominant blacks.

But some of the selected "dappled chestnut" ponies might be heterozygous for *E^D*, of the genotype *B–E^{D}eS–*. Among their descendants there would arise individuals of genotype *B–eeS–*, which would have white mane and tail with *red* body color rendered a little off shade by traces of fawn associated with it. These would be designated sorrel. They would not show dappling, which does not occur in a red pigmented body for the reason that there is not enough black pigment left in the body region to develop the dapple pattern. But they would be capable of transmitting dappling to their offspring, if the other parent supplied *E* or *E^D*, as in a cross with black.

If ponies of the selected genotype *B–E^D–S–*, were mated together, there would arise the genotype *B–E^D–SS*, in which the modifying gene is homozygous. If gene *S* is more effective in double than in single dose, such ponies would be a lighter shade than heterozygotes of genotype *B–E^D–S–*. An example of an *SS* silver dapple is probably found in Dr. Munn's mare "Peggy Ann," which produced only dappled colts, six in number, in matings with black stallions. Another well demonstrated example is the stallion Magic B, which when bred to black mares produced 11 silver dapple colts, and one recorded as "brown," possibly sorrel of genotype *B–eeS–*.

Experienced breeders of silver dapple ponies prefer to follow the type of mating employed in the production of Chestnut, the foundation sire, i.e., to breed a sorrel or a silver dapple to an intense black *B–E^D–* mate. The result is production of equal numbers of silver dappled ponies of genotype *B–E^D–S–* and of black ponies of genotype *B–E^D–*. All the dappled ponies will be heterozygous for *S*, the desired dark type with strong contrast between dappled body and white mane and tail; none of the blacks will carry *S*, so will be of full intensity.

We can now understand why the bay variety, once common among Shetland ponies, has become rare. If dominant black has by selection been made prevalent in the best families of black Shetlands, and was from that source transferred by Prince of Wales to Chestnut, the foundation sire of all silver dapples, we can see how *E^D* might become the prevailing *E* allele, not only among dappled chestnuts, but also in other varieties with which dapples were intercrossed. This would cause gradual suppression or concealment of the bay gene *A*, and bays would become scarce, since they can be recognized only in the genotype *A–B–E–*, not in *A–B–E^D–*.

Gray-White Ponies

The silver dapple mutation (*S*) has taken part in producing a new variety of white pony peculiar to Shetlands and commonly called either simply white, or gray-white.

By an accidental outcross or by mutation within pure bred Shetlands, the dominant gene for gray (*G*), which is well-known in Arabian and Percheron horses, has become established among Shetlands. Percheron horses are born black; in subsequent moultings of the coat, white hairs appear among the black, and these become more numerous at each successive moult, until the coat may become nearly or completely white in old animals. The gray mutation may

thus be described as one which causes progressive whitening of a black pigmented coat.

When by crosses of silver dapple with gray Shetlands, genes *S* and *G* became associated, it was found that the graying of a black pigmented coat began earlier and might even be found in the first coat (at birth) and would thus be permanent, throughout life, but colts of genotype *B–E–S–G–* were more often born with incomplete whitening of the coat. As white becomes increasingly evident, they are designated gray-white, and as yearlings or two-year-olds they are entirely white except for the skin (where visible on nose or around the eyes) and on the hoofs. See Figure 11.

A majority of the colts born white have brown eyes and blue skin, and this is considered the most desirable type. But a certain number have blue eyes and pink skin. These are commonly discarded, or outcrossed and are not registered. Their resemblance in eye and skin colors to the albinos of Palomino breeding suggests that they may be of brown (*bb*) rather than of black (*B–*) genotype.

The most desirable genotype of dapple chestnut pony is probably *A–* (or *aa*) *B–E^D–S–*, which seems to have been that of the foundation sire, Chestnut. If *E* is substituted for *E^D*, the coat becomes lighter, and the contrast between colored body and white mane and tail is diminished. Also the combination *A–B–E–S–* would result in a fully expressed bay pattern, and the mane and tail might become "silver" where the bay pattern would permit some red hairs to develop in mane and tail. Substituting *b* for *B* would have a similar lightening effect but more extreme, since brown rather than black pigment would undergo dilution.

Substitution of *ee* for *E^D* would result in a red body color, sorrel, which in the genotype *aaB–eeS–* would be uniform red including mane and tail, but in the genotype *A–B–eeS–* would have white mane and tail.

Substitution of *SS* for *S* would probably increase the diluting effect of *S* on black-brown pigment, and might make the body color undesirably light. However, it seems to have been satisfactory in the stallion Magic B.

Summary

1. The "chestnut" color of dappled chestnut Shetland ponies is not the equivalent of chestnut in horses. The coat of a chestnut horse is essentially brown pigmented, its genotype being *bb*; the coat of a dappled chestnut Shetland pony is essentially black pigmented, its genotype being *B–S–*.

2. *S* is a dominant diluting gene which reduces black pigment to a shade of dark cream, or light chocolate. It appears to have originated by mutation about the year 1886.

3. Four principal genes are concerned in the production of silver dapple ponies. They are with their alleles *A, a; B, b; E^D, E, e;* and *S, s*. Their effects in various combinations are discussed.

4. Dominant black *B–E^D–*, is an important constituent of the most desirable type of dappled chestnut, since it results in a maximum intensity of the black pigmentation. If *E^D* is replaced by *E*, the contrast between body color and white mane and tail would be diminished. Replacement of *B* by *b* would lighten the coat still more and change it qualitatively from diluted black to diluted brown.

5. Sorrels produced in the breeding of dappled chestnut ponies are not themselves dappled, but may produce dapples in crossings to a black mate. Their genotype is either *A–B–eeS–* or *aaB–eeS–*. The first sort have a red body color with white mane and tail; the second sort are red all over including mane and tail.

6. *S* when homozygous, as in the genotype *B–E^D–SS*, is probably more effective in diluting black, than when it is heterozygous, as in *B–E^D–Ss*.

7. The relation of the mutant gene *S* of Shetland ponies to the gene *D* of Palomino and buckskin horses is as yet uncertain. Both modify the intensity of black-brown pigment more than that of yellow-red pigment. The effect of *S* in modifying black is certainly greater than that of *D*, perhaps it is also greater in modifying brown pigment. Whether the two are alleles or analagous mutations of different genes remains to be determined.

8. Gene *S* in association with gene *G* accelerates the whitening effect of *G*, so that an *S–G–* colt may be born white or become so at an age of one or two years. A pony of this sort is known as gray-white.

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SHORT FIRST INTERNODE IN MAIZE

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GERMINATING corn emerges by growth of the first internode or mesocotyl and the coleoptile. Inge and Loomis[2] have shown that the mesocotyl is the structure which compensates for depth of planting. It continues growth until the coleoptile emerges after which its further elongation is inhibited. Thereafter growth proceeds by development of successive leaves which emerge from the protective coleoptile.

Recent studies concerning the influence of planting depth and temperature on stands of sweet corn[1] support the view that length of the first internode is a function of planting depth. The mesocotyl of six sweet corn strains planted one inch deep averaged 0.81 inch compared to 3.27 inches when planted four inches deep. However, one of the inbreds, line 5002, hereafter referred to as line *2* for simplictiy, involved in these trials consistently developed a short first internode averaging only 2.07 inches at the four-inch planting depth. To eliminate the possibility that this character was environmentally induced by frost or other injury, seed of the same strain produced in different years was germinated and found to behave in a similar way. This paper is a report on studies of the inheritance of the short first internode character. It behaves as a Mendelian recessive and the symbol *sfi*** *is* suggested. The gene has no apparent effect on elongation of internodes other than the first.

Comparatively deep planting is a prerequisite for the expression of short first internode. With shallow planting the coleoptile emerges as it does in wheat, barley and rye. In the experiments herein reported plantings were made at a depth of four inches. At this depth the coleoptilar or crown node of line *2* seedlings remains deeper than that of normal seedlings because of the inability of the first internode to grow to the indicated length. Consequently the coleoptile is unable to emerge and is ruptured while still underground by successively developing leaves which also usually fail to emerge. Stands of inbred *2* averaged only 49 percent at the four-inch planting depth as compared with an average of 80 percent for five normal lines (Table I). At normal planting depths in the field line *2* produces poor stands and the cause is overlooked unless the seedlings are dug and examined.

Short first internode seedlings exhibit three anomalies which are illustrated by line *2* plants in Figure 12. Seedlings which emerge after deep planting have short first internodes approximately two inches long as compared with an average normal length of 3.42 inches (Table II). In addition the first internode and coleoptilar node often are thickened and misshapen (Figure 12*A*). If the coleoptile fails to emerge successive seedling leaves grow horizontally underground (Figure 12*B*). They are contorted and etiolated. Superficially they resemble other seedling abnormalities reported in maize.[3, 4, 6]

A number of F_1 hybrids involving inbred *2*

TABLE I. Stand of short first internode of line 2 compared with average stand of five normal lines at two planting depths 20 days after planting

Strain	Planted 1 inch deep %	Planted 4 inches deep %
Inbred 2	71	49
Average of five normal lines	89	80

TABLE II. First internode length of sweet corn inbreds and F_1 hybrids when planted four inches deep. Figures represent averages for seedlings obtained from 100 seeds

Strain		First internode length
		Inches
Inbred	3742	3.35
	3647	3.80
	T1874	3.52
	P39	2.99
	Average	3.42
Inbred	2	2.00
F_1 hybrid	T1874 × 2	3.14
	2 × G.g.g.	2.69
	P39 × 2	2.67
	Average	2.83

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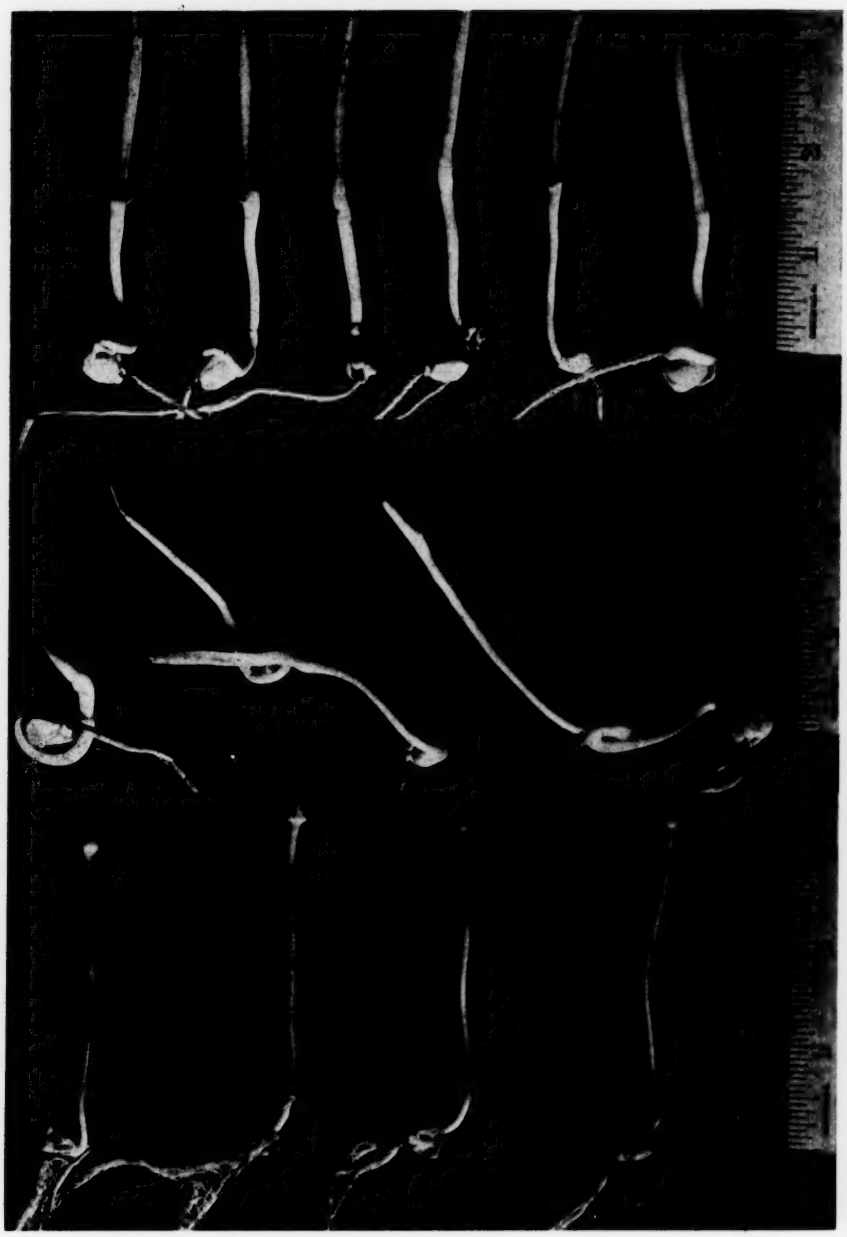
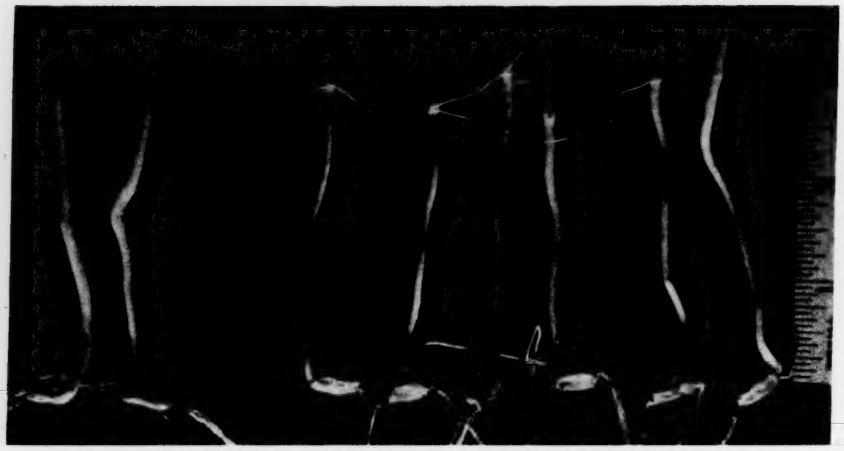
**Credit is due Walton Galinat for suggesting this symbol.



LINE 2 SEEDLINGS COMPARED WITH NORMAL

Figu e 12

A—Seedlings which chanced to emerge, showing short, thick first internodes; *B*—Contorted seedlings which failed to emerge; *C*—Normal P-39 seedlings.



SHORT FIRST INTERNODE AND NORMAL SEEDLINGS
Figure 13

Segregations from an F_2 hybrid progeny. *A* shows *sfi* and *B* shows normal seedlings.

were found to have first internodes averaging 2.83 inches (Table II), a length somewhat shorter than that of the normal lines reported in these investigations.

Twenty-five F_2 progenies involving line 2 were classified for length of first internode (Figure 13) and these data are summarized in Table III. While several progenies deviate significantly from the expected 3:1 ratio, assuming that the *sfi* character is controlled by a single recessive gene, the total for all progenies showed a close fit with a chi square of 0.185 and a recessive class of 544 compared with an expected of 553. Expression of the *sfi* gene as indicated by average first internode length for the recessive class (Table III) varies to some extent in the different F_2 progenies. Linkage relationships of *sfi* have not been determined.

Studies regarding the expression of short first internode during germination in total darkness and the associated production, distribution or inactivation of auxin[5] may lead to further understanding of this gene.

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TABLE III. Short first internode and normal seedlings in segregating F_2 progenies tested for goodness of fit for a 3:1 ratio

Progeny	+	*sfi*	Total	% *sfi*	χ^2	Average first internode length for recessive class
2 × Gcg	79	19	98	19.4	1.10	2.37 ± .44‡
2 × 5125-2	65	31	96	32.3	2.72	2.35 ± .26
P39 × 2	55	32	87	36.8	6.44*	2.22 ± .30
1o × 2	56	15	71	21.1	0.56	2.18 ± .31
2 × TRR	74	19	93	20.4	1.03	1.93 ± .29
2 × Tend	67	25	92	27.2	0.23	2.12 ± .16
2 × 5125-1	72	22	94	23.4	0.12	2.13 ± .13
2 × 39wx	66	25	91	27.5	0.29	2.15 ± .16
2 × 2730	76	16	92	17.4	2.84	1.97 ± .69
2 × Sug	78	19	97	19.6	1.51	2.46 ± .30
2 × 3740	65	30	95	31.6	2.19	1.93 ± .11
2 × 2508	69	20	89	22.5	0.30	1.96 ± .20
2 × 32wx	81	18	99	18.2	2.45	1.82 ± .30
2 × 11934	72	24	96	25.0	0.00	1.87 ± .26
2 × 3742	84	12	96	12.5	8.00†	1.69 ± .16
2 × Gcg-3	62	23	85	27.1	0.19	1.77 ± .30
M13 × 2	64	27	91	29.7	1.05	1.80 ± .23
2 × Sup	36	20	56	35.7	3.42	1.50 ± .18
2 × loc-3	63	24	87	27.6	0.31	1.72 ± .30
32 × 2	53	17	70	24.3	0.01	1.57 ± .28
3647 × 2	79	20	99	20.2	1.21	2.09 ± .20
P39 × 2	66	21	87	24.1	0.00	1.58 ± .20
2 × Sup	62	16	78	20.5	0.83	2.22 ± .24
2 × Gcg-1	64	28	92	30.4	1.44	1.93 ± .13
Td × 2	64	20	84	23.8	0.06	1.33 ± .23
Total	1667	544	2211	24.5	0.18	
Expected	1658	553	2211	25		
Average						1.95

* χ^2 significant at 0.05.
† χ^2 significant at 0.01.
‡ Standard deviation of a single observation.

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A COMPARISON OF THIAMINE, NIACIN, AND RIBOFLAVIN CONTENT

In Diploid and Tetraploid Plants

FELIX G. GUSTAFSON*

IN the search for larger and better plants geneticists and plant breeders have gone to considerable labor to increase the number of chromosomes in their plants. It was shown, many years ago, that some of these plants with an increased number of chromosomes were larger, had larger flowers and fruits, than those with fewer chromosomes.

Although in many instances the tetraploid plants are more vigorous than the diploid, they are not necessarily superior in all respects to the plants with fewer chromosomes. This has been well illustrated with respect to the growth hormone content by Avery and Pottorf[2] and Gustafson,[6] who found that the diploid plants were much richer in growth hormone than were the autotetrapolid plants.

The vitamin content of polyploids has received considerable attention, but there is no uniformity in the results. Thus Sansome and Zilva[14] reported a higher ascorbic acid content in the autotetraploid tomato plants than in the diploid plants, whereas Key[8] found no difference between diploid and tetraploid tomatoes. Pyke and Melville[13] studied the ascorbic acid content in the flesh of the hips of a number of species of *Rosa* and they found a great difference in the vitamin content. The species indigenous to Scotland and northern England had a much higher concentration than those commonly found in the south of England. Darlington,[5] checking on the chromosome number of these plants, found that the ones that had a high vitamin C content also had a high chromosome count, whereas those with low vitamin content had a lower chromosome number. Barr and Newcomer[3] determined the vitamin C content of cabbage and reported that the autotetraploid had a 23.86 percent higher concentration than the diploid, but in a personal communication Barr states that in later work this has not been substantiated. Smith and Olmo[15] found no essential difference in the pantothenic acid and riboflavin content in diploid and autotetraploid grapes. Noggle[11] observed that the concentration of ascorbate in fresh tissue of Rosen rye did not show any consistent relationship to the tetraploid condition, but on the dry weight basis the tetraploids contained a higher concentration than the diploids. There was no consistent difference in niacin in the two plants. The riboflavin content of the tetraploids was generally equal or greater than that of the diploids. Later Noggle[12] states that on the basis of fresh weight the ascorbic acid was higher in the diploid plants than in the tetraploid plants, but on the basis of dry weight the reverse was true.

Interest in the vitamin content of green plants is currently quite great. The regulation of vitamin content by controlling the mineral nutrition or other environmental factors does not seem generally feasible. If the tetraploid condition was found to be accompanied by a consistent difference in vitamin levels, such a condition could be readily exploited. The availability of diploid and autotetraploid forms of three species and an additional pair of unknown relationship permitted this possibility to be investigated. The thiamine content, not

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previously studied in this connection, was included with the riboflavin and niacin.

Materials and Methods

The material used includes whole young plants and leaves with stem tips (1 to 2 cm. long) of *Lycopersicum pimpinellifolium* (currant tomato); young leaves with stem tips and mature leaves but no stem of *L. esculentum* var. ex-haploid; stem tips with leaves or only leaves of *Vinca rosea*, when plants were in blossom; young leaves or leaves and stem were used from *Tradescantia ohiensis*. The ex-haploid tomato and *Vinca* have been propagated by cuttings. The *Tradescantia* was brought into the greenhouse from the field. The *L. pimpinellifolium* has been grown from seeds. All of the plants were grown in the greenhouse, whether used in summer or winter. The experiments have extended over a period of several years so that the growing conditions have been quite varied.

The vitamins, thiamine, riboflavin and nicotinic acid (niacin) have been determined in the above mentioned plants. The two former were determined chemically according to the method of Connor and Straub[4] as adapted for green plants by Gustafson.[7] The nicotinic acid has been determined by the microbiological method as outlined in the Methods of Vitamin Assay,[1] with some minor changes necessitated by the material used. The end point in the titration is determined by a pH meter instead of colorimetrically.

Being well aware of the fact that vitamin content is not the same in all parts of the plants, that it varies with the season and with the age of the plant, attempts have, for the most part, been made to make comparative analyses at different times of the year and in all instances the material was carefully selected so as to use only comparable parts of plants of the same age. Sampling was always done with care to insure that in all cases the comparison between the diploid and tetraploid plants would be valid. Vitamin contents were expressed on the green weights of the whole sample as collected.

Results

Lycopersicum pimpinellifolium. In the spring of 1949 several analyses of young plants were made of this species. The plants ranged in age from 58 to 67 days. When grown in the greenhouse the young tetraploid plants had larger leaves and were a little taller than the diploid plants; in the field these differences were also apparent initially, but as the plants grew older the differences disappeared and there was no difference in the size of the fruits. In the first three samplings comparable whole plants were used; but in the fourth experiment the plants available were large enough so that only the apical part was used. Again in January 1951 some more analyses were made on 79-day-old plants. From these plants only leaves and stem tips were used. In this experiment the analyses were made in four replicates, which gives better results. When all data on this plant were statistically analyzed it was found that the difference in riboflavin in the diploid and tetraploid was significantly higher at the one-percent level in the diploid plants, but the difference in thiamine and niacin was not significant (Table I).

Lycopersicum esculentum var. ex-haploid was originally obtained from E. W. Lindstrom, who developed both the diploid and tetraploid plants from a haploid plant. The tetraploid plants set fruit very rarely, probably due to poor pollen development and to a short style which does not reach up to the stamens. The diploid is fertile, but as the tetraploid does not produce seeds, both have been propagated by cuttings. As shown in Table II, the work was done at different times of the year with both young and ma-

TABLE I. Vitamin content in diploid and autotetraploid *Lycopersicum pimpinellifolium* plants (vitamin content is given as micrograms per gram fresh weight of plant).

Exp. No.	Date	Riboflavin 2n	Riboflavin 4n	Thiamine 2n	Thiamine 4n	Niacin 2n	Niacin 4n	Remarks
200	5-17-49	1.86	1.40	1.38	1.11	6.30	6.08	Whole plants, in blossom, 58 days old
201	5-19-49	1.87	1.52	0.99	1.13	7.40	6.50	Whole plants, in blossom, 60 days old
202	5-24-49	1.86	1.41	0.94	1.24	6.08	5.97	Whole plants, in blossom, 65 days old
203	5-26-49	1.43	1.25	0.90	1.31	7.46	7.68	Base of plants not used. 67 days old.
336	1-15-51	1.73	1.47	1.31	1.02	6.43	5.83	
		1.66	1.47	1.25	1.00	6.32	5.73	Plants 79 days old; only leaves and
		1.38	1.41	1.13	0.97	6.30	5.75	tips of plants used. 4 plants for each
		1.50	1.30	1.24	0.81	6.38		lot.
Unweighted mean of means		1.66	1.40	1.12	1.07	6.58	6.16	
		$P < 0.01$*		$P > 0.20$		$P > 0.10$		

*The difference between the riboflavin content of the 2n and 4n plants was tested by an application of the analyses of variance. There were two criteria of classification, chromosome number and dates of sampling with unequal numbers of replication. The data for 5-17, 5-19, 5-24 and 5-26 were treated as a set of four replications. As a result of this analysis it was found that the effect due to the number of chromosomes was significant at the one-percent level. The data for the other vitamins and the other plants have been treated in the same way.

ture leaves, and in some instances stem tips one to two inches were also included. The material has varied considerably in the different experiments and so has the time of year when the analyses were made. The results obtained should therefore represent the typical conditions found in this plant. Statistical analyses of the data indicate that there is no significant difference in the thiamine, riboflavin and niacin content of diploid and tetraploid plants.

Vinca rosea is a perennial plant as grown in the greenhouse. Neither the diploid nor the tetraploid plants produce fruits or seeds, though they are in continuous blossom. They have therefore been propagated by cuttings. The tetraploid plants have both larger leaves and flowers and in other respects are more vigorous than the diploids.

The determinations reported here were made at various seasons over a period of three years. As noted in Table III, a short apical piece of the branches with its leaves was used, except in the last two experiments. An analysis of the variance shows that the difference in riboflavin and niacin between the diploid and tetraploid plants is significant at the one-percent level, but the thiamine differences are not significant.

Tradescantia ohiensis was collected in the field near Ann Arbor, Michigan, by Dr. Donald Dean. He determined the chromosome number and found that in the same locality both diploid and tetraploid plants were present. Whether the tetraploids are auto- or allotetraploids cannot, of course, be stated. These plants were cultured in the greenhouse and the writer obtained some representative plants. The tetraploids were, on the whole, a little more robust than the diploids. The analyses are limited both in number of determinations and distribution through the season. Even though the material used was quite different in stage of development, yet the greater concentration of riboflavin in the diploid is significant at the one-percent level. The difference in thiamine and niacin is not significant, though all analyses are in favor of the diploid plants.

Discussion

As reported in the literature, the relation between the chromosome number of plants and their vitamin content is not very consistent, but with only a few exceptions the vitamin content has been found to be higher in the tetraploid or the same as that in the diploid plants. Noggle[12] reported that in the Rosen rye the diploids had a higher riboflavin content, on the basis of fresh weight, than the tetraploids.

In the present investigation, *Vinca rosea, Tradescantia ohiensis* and *Lycopersicum pimpinellifolium* have been shown to have a higher riboflavin content in the diploids than in the tetraploids. In *L. esculentum* var. ex-haploid there were no significant differences. *V. rosea* has also a higher niacin content in the diploids, but in the other plants there is no essential difference in niacin content. Thiamine is essentially the same for diploids and tetraploids in all four species studied. These results are at variance with those reported in the literature.

In his review Noggle[10] notes variations in all of the physiological factors studied; in some species the tetraploid plants may have a higher concentration of a particular substance and in other

TABLE II. Vitamin content in diploid and autotetraploid *Lycopersicum esculentum* var. ex-haploid plants (vitamin content is given as micrograms per gram fresh weight of plant).

Exp. No.	Date	Riboflavin 2n	Riboflavin 4n	Thiamine 2n	Thiamine 4n	Niacin 2n	Niacin 4n	Remarks
227	10-17-49	2.30	2.21	1.24	1.35	10.41	9.23	Apical tips with immature leaves
231	10-31-49	2.34	2.38	1.58	1.40	8.54	7.70	
232	11- 2-49	2.08	2.13	1.14	1.08	8.50	7.31	Young laterals stem and leaves
233	11- 7-49	2.40	2.18	1.47	1.62	8.09	8.32	Apical and lateral stem tips with immature leaves
234	11- 9-49	2.28	2.10	1.50	2.02	7.98	7.28	
236	11-16-49	2.37	1.92	1.25	1.54	7.60	6.27	Mature leaves from plants 233 & 234
424	3- 5-52	1.64	2.00	1.01	1.13	6.73	7.59	Whole leaves, young and mature, no stem
		1.82	1.92	1.14	1.21	7.55	7.45	
		1.82	2.00	1.05	1.25	7.53	8.29	
442	7- 7-52	1.26	1.07	0.78	0.74	10.09	9.88	
		1.26	1.10	0.72	0.75	10.14	9.95	Young leaves from young and old plants
		1.25	1.06	0.74	0.77	9.91	10.21	
		1.21	1.13	0.71	0.82	9.94	10.45	
Unweighted mean of means		1.767	1.739	1.078	1.156	8.603	8.528	
		$P > 0.05$		$P > 0.20$		$P > 0.20$		

species the diploids. Growth hormones as mentioned earlier have been found to be more concentrated in the diploid plants than in tetraploids.

There is, of course, no reason why one should expect that the doubling of the chromosome number in a plant should also increase its capacity for vitamin synthesis. The morphological differences that have been observed to occur, when the chromosome number is increased in a plant, are mainly due to the increase in size of the cells.[9] This in many cases is mainly due to an increased water content, rather than to an increase in protoplasm.[10] Relatively few diploids and tetraploids have been subjected to comparative analyses. These investigations in general have been limited to those pairs in which the tetraploids were larger or in other ways looked different from their fellows, or were produced artificially. Not all polyploids are larger than plants with a lower chromosome number. Several years ago the writer had some autotetraploid snapdragons that were morphologically indistinguishable from the diploid plants from which they had originated, and were found only because chromosome counts were made.

It certainly is not established that doubling the chromosome number is an effective or dependable means of improving the quality of a plant. Appropriate hybridization holds greater possibilities. For example, *L. pimpinellifolium* has a fruit with a high vitamin content but small in size. Crosses have been made between this variety of tomato and Rutgers, which has a large fruit that is, however, low in vitamin content. Table V gives the vitamin content in the parents and the hybrid. Three

TABLE III. Vitamin content in diploid and autotetraploid *Vinca rosea* plants (vitamin content is given as micrograms per gram fresh weight of plant).

Exp. No.	Date	Riboflavin 2n	Riboflavin 4n	Thiamine 2n	Thiamine 4n	Niacin 2n	Niacin 4n	Remarks
201	5-19-49	3.98	3.44	0.77	0.62	7.94	6.03	2 in tips with leaves
202	5-24-49	3.91	3.26			6.45	6.01	as in 201
203	5-26-49	3.58	3.52	0.82	0.78	8.64	7.71	as in 201
294	7-19-50	3.49	3.48	0.91	1.04	7.78	7.30	
		4.04	3.40	0.94	0.92	8.41	6.98	as in 201
		4.02	4.02	0.87	0.70	8.08	7.14	
310	9-25-50	3.43	3.10	0.92	1.02	10.88	8.32	
		3.70	3.20	1.01	0.77	10.06	8.12	2 in tips with 2 or 3 pairs of leaves
		3.64	3.34	0.80	0.60	10.88	8.81	
337	1-17-51	3.70	3.34	0.74	0.58	7.94	6.61	
		3.64	3.16	0.88	0.57	8.25	6.18	
		3.75	3.25	0.86	0.59	8.22	6.31	as in 310
		3.62	3.36	0.66	0.57	8.33	6.94	
367	7- 9-51	3.48	4.04	1.15	1.32	10.80	9.10	
		3.08	3.68	1.12	1.27	9.75	9.33	
		3.64	3.48	1.10	1.25	10.73	8.50	as in 310
		3.24	3.18	1.06	1.34	10.63	9.02	
401	12- 5-51	4.45	3.69	0.34	0.45	11.1	7.3	
		4.71	3.57	0.35	0.49	9.9	7.2	as in 310
		3.88	4.09	0.35	0.43	9.7	7.8	
431	3-31-52	4.20	3.49	0.64	0.60	12.50	10.15	
		3.92	3.53	0.68	0.56	12.56	10.15	as in 310, but these plants were not
		3.90	3.49	0.74	0.44	12.12	9.73	as healthy as usual
		4.12	3.64	0.80	0.57	12.25	10.33	
441	7- 2-52	3.63	3.54	0.56	0.57	12.18	9.93	
		3.32	3.10	0.54	0.51	12.44	10.19	only leaves used, 2 prs. mature and
		3.27	2.82	0.56	0.51	12.04	9.95	1-3 young leaves
		3.20	2.75	0.48	0.52	12.15	10.13	
458	9-29-52	5.84	5.06	1.34	1.10	11.56	10.10	
		5.60	4.52	1.42	1.28	11.44	9.97	3 prs. of leaves used from each tip
		5.74	5.06	1.28	1.32	12.08	10.30	
		5.88	4.70	1.40	1.42	11.47	10.45	
Unweighted mean of means		3.978	3.593	0.829	0.785	10.163	8.388	
		$P < 0.01$		$P > 0.05$		$P < 0.01$		

different selections of *L. pimpinellifolium* and two of Rutgers were used. Except for the niacin in two selections the hybrid fruits have a higher vitamin content than the Rutgers parent. The hybrid fruits were still small and further crossings would have to be made in order to get a marketable fruit.

Summary

Riboflavin, thiamine and niacin have been determined in diploid and tetraploid plants of *Vinca rosea, Lycopersicum pimpinellifolium, L. esculentum* var. Ex-haploid and *Tradescantia ohiensis.*

It has been found that the riboflavin content is significantly higher in the diploid plants of *V. rosea, L. pimpinellifolium* and *T. ohiensis* than in the tetraploids. Niacin is also higher in the diploids of *L. pimpinellifolium.* Otherwise there is no significant difference between the diploid and tetraploid plants in any of the species studied. These results do not support the view that polyploidy might be a means of obtaining tissue of higher vitamin content. Appropriate hybridization remains a more probable device.

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TABLE IV. Vitamin content in diploid and tetraploid *Tradescantia ohiensis* plants (vitamin content is given as micrograms per gram fresh weight of plant).

Exp. No.	Date	Riboflavin 2n	Riboflavin 4n	Thiamine 2n	Thiamine 4n	Niacin 2n	Niacin 4n	Remarks
432	4- 2-52	0.75	0.64	0.09	0.00	1.95	1.68	Whole plant just through blossoming
438	5- 5-52	0.80	0.53	0.21	0.09	2.91	2.81	Plants past blossoming
443	7- 9-52	1.54	1.18	0.42	0.38	3.95	3.35	
		1.64	1.26	0.43	0.40	3.87	3.67	2 pots of 2n and 3 pots of 4n, but probably several plants in each pot. Plants healthy, green leaves used.
		1.42	1.26	0.35	0.28	3.48	3.35	
		1.38	1.36	0.35	0.40	3.73	3.69	
Unweighted mean of means		1.135	0.925	0.269	0.205	3.094	2.880	
		$P < 0.01$		$P > 0.10$		$P > 0.20$		

TABLE V. Vitamin content in tomato fruits-Rutgers, *L. pimpinellifolium* (Currant) and hybrid between the two (vitamin content is given in micrograms per gram fresh fruit).

Vitamin	Exp. No.	Rutgers	Hybrid	Currant	Remarks
Thiamine	297	0.76	1.06	1.10	green fruits
		0.76	1.02	1.54	" "
	305	0.75	0.84	0.86	ripe "
	307	0.72	0.77	1.54	" "
		0.72	1.00	1.04	" "
Riboflavin	297	0.32	0.57	0.82	1292
		0.32	0.48	1.43	1796
	305	0.15	0.24	0.37	Ortega (Currant)
	307	0.11	0.26	0.58	1796
		0.11	0.18	0.35	1292
Niacin	297	2.82	2.87	3.7	*
		2.82	3.12	4.81	*
	305	4.40	5.60	6.04	†
	307	5.07	4.37	7.77	*
		5.07	4.50	6.15	*

*The seeds from which these plants were grown were supplied by Dr. Mark L. Tomes, Purdue University.
†The seeds were supplied by Dr. H. H. Munger, Cornell University.

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RACE CROSSING IN PARADISE?

THE town of Calw in Southern Germany has played an important part in the beginnings of genetics. Here J. C. Kölreuter (1733-1806) carried out some of his work which established firmly the existence of sexuality in plants and resulted in the first controlled production of plant hybrids, and here K. F. Gärtner (1772-1850) experimented for over 25 years in Kölreuter's tradition and became one of the leading plant hybridizers before Mendel.

These men were members of a group of the many distinguished pharmacists and physicians of this region who, as accomplished amateurs, made fundamental contributions to scientific knowledge. The Gärtner family was associated closely with the Alte Apotheke, Calw's pharmacy. When, in 1692, its building was destroyed by Louis XIV's army, Achatius Gärtner (1662-1728) was charged with the erection of a new structure. It still stands, serving its old purpose.

Inside, the modern student of race finds a curious symbol. It is a painting of Paradise on a door panel, probably dating to around 1710.* There is the lion and the lamb and other animals which peacefully share the idyllic landscape. Several tailed monkeys are seen up in the trees and a strange tall ape, whose erect posture suggests that the painter had never encountered one of its kind alive, stands in the background. The main interest is centered on Adam and Eve. Skillfully, the first human male and female are depicted much as in other, greater compositions. One contrast, however, is unique in this panel. Adam is a white man, Eve a black woman. It seems that the pious unknown artist of the century of Rationalism—or was it his patron?—had contemplated upon the existence of different races of mankind. Must not their origins go back to the stem parents? Thus, the painter placed into Paradise a member each of the most strikingly different human varieties to be the progenitors of the still existing diversity.

A white Adam and black Eve seem to be unknown in other art. True, in representations of the continents a white and a black, a yellow and a red figure has often been used. But this painting of Paradise is not a symbol of the two continents nor are the same artist's works on the adjoining panels related to geography. They show the sources of materials used for the production of drugs: a pharmacist's garden, a seascape with whales and fishes, and a mine as the furnisher of minerals and chemicals.

The tolerance of the eighteenth century has left many noble reminders in the intellectual and political history of the Western World. The humble picture in the Calw pharmacy impresses us as another part of this heritage.

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*In a letter to Prof. E. Lehmann, Tübingen (Feb. 12, 1953) the present owner of the pharmacy, Mr. F. C. Reichmann, gives the probable building date of the annex which houses the door panel as 1710. Lehmann, in his book "Schwäbische Apotheker und Apothekergeschlechter in ihrer Beziehung zur Botanik," Stuttgart: Hempe Verlag, 1951, earlier had estimated the origin of the painting as around 1730.

EIGHTEENTH CENTURY PARADISE
Figure 14

The garden of Eden, complete with the lion, the lamb and the biological basis for the racial diversity of human times is depicted by an unknown artist working about 1710.

HERITABLE CHARACTERS IN MAIZE

Description and Linkage of Brittle Endosperm-2

HOWARD J. TEAS AND ANNA N. TEAS*

IN 1926 Mangelsdorf[4] described a recessive endosperm mutation which he named "brittle" because of the endosperm texture. A second factor for brittle endosperm has been included in the Maize Genetics Cooperation stocks since at least 1934 as brittle endosperm–2 (brittle–2, designated by the symbol bt_2). In their summary of maize linkages in 1935, Emerson, Beadle and Fraser[2] listed brittle–2 as having been found by Dr. G. F. Sprague. The brittle–2 chromosome was not identified and no published reference to the character was cited.

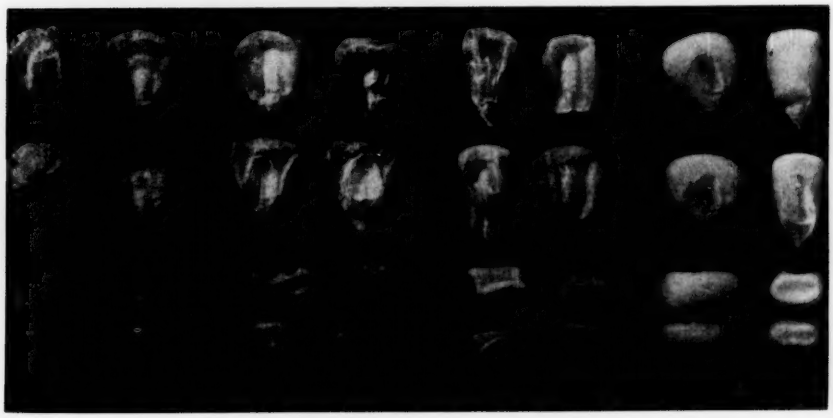
In 1949 seed of brittle–2 was obtained from the Maize Genetics Cooperation at Cornell University. Brittle–2 plants were crossed to standards, to homozygous sugary–1 (su_1), and to homozygous brittle–1 (bt_1). The F_1 kernels confirmed that the brittle–2 character is recessive to the normal and not allelic with bt_1 or su_1 (su_1 is hereafter referred to without the subscript). In 1951 a homozygous brittle–2 plant that had been pollinated by translocation B–4a produced an ear on which approximately half of the kernels were brittle. In translocations between a B chromosome and an ordinary chromosome there is nondisinjunction of the B centromere at the division of the generative nucleus of the pollen, giving rise to two unequal nuclei.[6] Since one of these nuclei goes to the embryo and one to the endosperm there is the possibility of linkage detection by a hemizygous test.[6,7] The occurrence of brittle kernels on the ear pollinated by translocation B–4a therefore indicated that the gene for brittle–2 was located in the portion of the short arm of chromosome 4 attached to the B chromosome centromere. The following year seven plants homozygous for brittle–2 were crossed with plants carrying translocation B–4a. In all seven cases the ears had approximately half of the kernels brittle. These results confirmed the earlier cross. The heterozygous B translocation pollen parents were tested by crossing onto homozygous sugary plants where ears showing about one-half sugary kernels were obtained. Inasmuch as *su* is located in chromosome 4 distal to the break in translocation B–4a,[6] these crosses verify the constitution of the translocation stock and definitely place bt_2 and *su* in the same chromosome arm.

Since *su* is in the proximal portion of the translocated chromosome 4 arm, near the break,[6] F_2 segregations for *su* and bt_2 were checked for further evidence on the location of the bt_2 factor. F_1 plants from a cross of sugary by brittle–2 were selfed and the F_2 kernels classified for the normal and two mutant phenotypes. If bt_2 were completely linked to *su*, a ratio of 1 normal kernel (heterozygous for both *su* and bt_2) to 1 mutant (half of them sugary, half brittle) would be expected. However, the short arm of chromosome 4 is known to have a genetic length of at least 71 crossover units distal to the *su* locus.[5] The possibility therefore existed that bt_2, although located in the short arm of chromosome 4,

TABLE I. Data obtained from counts of F_2 ears from sugary-1 × brittle-2

Pedigree number	Kernel phenotype		
	normal	sugary	brittle
1574-4	113	57	70
1574-5	50	19	20
1574-7	126	66	62
1574-8	124	54	65
1574-9	115	58	57
1575-7	17	7	7
Totals	545	261	281
Total normal vs mutant	545	542	

*Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California. This work was supported by funds from the Atomic Energy Commission administered through contract with the Office of Naval Research, United States Navy (Contract N6-onr-244, Task Order 5, Project NR-164-340). The authors are grateful to Dr. H. H. Smith for seed of brittle–2 and to Dr. H. Roman and Dr. E. G. Anderson for translocation B–4a stocks.



SUGARY, BRITTLE AND NORMAL KERNELS
Figure 15

Kernels of sugary (*A*), brittle-1 (*B*), brittle-2 (*C*) and normal (*D*).

might be in the distal third of the arm and show no linkage with *su*. If this were true, a ratio of 9 normal to 7 mutant kernels (total of brittle and sugary) would be expected in the F_2. Counts of kernels on the F_2 ears are shown in Table I. The totals, 545 normal to 542 mutant, indicate a close linkage of *su* and bt_2. No mutant kernels were noted that differed from either the sugary or brittle phenotypes in a way suggesting that they might represent the double homozygote. The linkage may be sufficiently close that no double mutant kernels occurred; however, it may be, as in segregations for bt_1 and *su*, that the double mutant so closely resembles the brittle phenotype as to make separation extremely difficult.

Endosperms of brittle-1 and brittle-2 both have a brittle texture, are shrunken, slightly darker in color than normal, and are intermediate in wrinkling and translucency between sugary and normal. Some of these characteristics can be seen in Figure 15. Relative weights of endosperms from segregating ears were: sugary, 75 percent; brittle-1, 46 percent; and brittle-2, 66 percent of normal. Germination of brittle-2 is variable, but tends to be poor. Like brittle-1 and sugary, brittle-2 seeds are more susceptible to fungus attack than are normal seeds on the same ear.

It was of interest to determine the extent of chemical changes associated with the morphological difference between brittle-2 and normal endosperm. Fat, nitrogen, and carbohydrate analyses were carried out on degermed, ground, vacuum-dried kernels. As seen in Table II, brittle-2 endosperm is higher than normal in fat, protein, reducing sugars, and sucrose; has about the same or less water-soluble polysaccharide (dextrin) content; and less starch. Also included in Table II are values[8] for the biologically interrelated substances tryptophan, niacin, and indoleacetic acid, which were higher in brittle-2 than normal. Inasmuch as sugary endosperm has also been reported to have higher fat, protein,[3] reducing sugar, sucrose,[1] tryptophan, niacin, and indoleacetic acid,[8] and lower starch,[3] the principal chemical difference between brittle-2 and sugary is the approximately normal water-soluble polysaccharide content of brittle-2 compared to the extremely high levels reported for sugary.[1]

The darker color of brittle-1 reported by Mangelsdorf[4] and noted here for brittle-2, was traced to ether-soluble yellow-orange pigment, not further characterized.

Summary

The factor for brittle endosperm-2 (bt_2) has been located in the short arm of chromo-

TABLE II. Chemical composition of normal and brittle-2 endosperm from segregating ears

Substance	Kernel phenotype					
	Ear 1		Ear 2		Ear 3	
	bt_2	normal	bt_2	normal	bt_2	normal
	%	%	%	%	%	%
Fat	7.6	1.6	6.6	1.2	7.7	1.7
Protein (N × 6.25)	18.5	17.6	14.9	12.9	17.7	15.1
Reducing Sugars	5.2	.7	8.5	.6	9.5	.7
Sucrose	2.7	.04	7.5	.01	2.0	.09
Water-soluble polysaccharides	.3	.7	.5	.7	.4	.7
Starch	36.2	65.3	25.7	57.6	38.4	51.2
	μg/gm	μg/gm	μg/gm	μg/gm	μg/gm	μg/gm
Tryptophan*	921.	349.	906.	322.	971.	301.
Niacin*	77.7	34.1	59.4	31.2	57.0	30.1
Indoleacetic acid*	92.0	40.0	74.3	37.0	84.0	35.5

* From Teas and Newton.[8]

some 4 closely linked with the factor for sugary endosperm–1. Endosperm of brittle–2, previously reported to have higher tryptophan, niacin, and indoleacetic acid than normal, also had higher fat, protein, reducing sugar, and sucrose; about the same amount of water soluble polysaccharide; and lower starch.

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Genetics in the U.S.S.R.

(Continued from page 132)

by Lysenko were published recently in *Agrobiologia*, 1950. English editions have been published by the Foreign Language Publishing House in Moscow, and distributed in India, where the senior author received a copy from Dr. P. Masheshwari of Delhi University. We have been unable to obtain a copy of this publication in the United States or Japan.

In this paper, Lysenko asserts—"It has been observed year after year when cultivating branched wheat (*Triticum turgidum*) on experimental plots of the Lenin Academy of Agricultural Sciences of the U.S.S.R., and in a number of other localities, that admixtures of soft and durum wheats, oats, 2- and 4-rowed barley and also spring rye appear in the crops. All our observations lead us to conclude that the original source of the admixtures was the branched wheat (*Triticum turgidum*) itself." In other words, the 28-chromosome *Triticum turgidum* had produced not only the 28-chromosome *Durum* wheats and the 42-chromosome bread wheats, but also the 42-chromosome genus *Avena*, the 14-chromosome genus *Hordeum*, and the 14-chromosome genus *Secale*. This direct transformation of species and genera is attributed to the environment, and Lysenko maintains that this transformation is continually occurring under suitable conditions. So far such "transformation" has been observed only in the U.S.S.R., and only by the "Michurin" biologists. The only rational explanation of Lysenko's observations is that there were some seeds of *Durum* and bread wheats, oats, barley, and rye in the *Triticum turgidum* seed which was planted, or that the various other species and genera were seeded in the experimental plots by accident or by design.

Lysenko concludes his article with the hope "that before long the development of the theory of Michurinist biology will make it possible to accumulate data also for zoological objects analogous to the data taken from the world of plants." By inference we might assume that under suitable conditions, perhaps by proper housing or diet, the Soviet scientists will be able to convert Orang-outangs into humans or vice versa.

In normal times a biologist confronted with such obviously inane statements would dismiss them as the product of an eccentric or moronic mind, but Lysenko's ideas are taken seriously in the U.S.S.R. and in Communist China. Many of Lysenko's fantastic claims are also accepted as facts by fiction writers who have invaded the field of science in the United States.

It is difficult to understand the motivation which has led Lysenko and his associates to make claims which are so obviously false. Perhaps they are suffering from a delusion complex in their attempts to make biological science conform with political philosophy, but it seems incredible that the realistic Soviet administrators would permit practices which can only retard the progress of agriculture in the U.S.S.R. At the same time both Soviet and Chinese Communists deny the validity of the Malthusian laws of population growth on the grounds that potential agricultural production is unlimited.

Perhaps these Soviet scientists are only following the practice of the political leaders who pretend to be more stupid and irrational than they really are. Such behavior of political leaders is not limited to the U.S.S.R., and seems to reflect the revolt of the masses against intelligence and rational thought.

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THE LOCATION OF 'TREMBLER' IN LINKAGE GROUP VII

Of the House Mouse

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THE dominant gene trembler (*Tr*) in the house mouse was not assigned to its linkage group when first described (Falconer[3]), tests for linkage having been made with only a few other loci. When a set of specially constructed linkage-testing stocks (Carter and Falconer[1]) were available tests were continued, and those with Stock I proved trembler to be linked with rex (*Re*) and so to belong to linkage group VII. The original tests were in the repulsion phase, but coupling data were later obtained, and together they show a recombination frequency of 23±2.6 percent. The results of these tests are given in Table I; all the matings were double backcrosses with the female as the segregating parent.

The next step was to determine the position of trembler with respect to the other known loci in linkage group VII. This group consists of the three genes rex, shaker-2 and waved-2, with recombination frequencies in females of about 22 percent between *Re* and *sh-2* (Falconer[2]) and 25 percent between *sh-2* and *wa-2* (Snell and Law[5]). In addition, vestigial (*vt*) has recently been shown by Michie[4] to lie close to *sh-2*, but since its order is not yet known this locus is not included in the tests with trembler. The observed recombination frequency of 23±2.6 percent between *Tr* and *Re* allows three possible positions for trembler, marked a, b and c below:

$$\underset{a}{\uparrow}\ \text{——}\ Re\ \text{——}\ \underset{b}{\uparrow}\ sh\text{-}2\ \text{——}\ \underset{c}{\uparrow}\ wa\text{-}2$$

In order to decide between these three possible positions, matings involving *Tr* with *sh-2* and *wa-2* were first made, thus

$$\frac{Tr\ +\ +}{+\ sh\text{-}2\ wa\text{-}2}\ ♀ \times \frac{+\ sh\text{-}2\ wa\text{-}2}{+\ sh\text{-}2\ wa\text{-}2}\ ♂ \quad \ldots\ldots\ (A)$$

Later it became possible to make the more efficient matings involving *Tr* with *sh-2* and *Re*, thus

$$\frac{Re\ Tr\ +}{+\ +\ sh\text{-}2}\ ♀ \times \frac{+\ +\ sh\text{-}2}{+\ +\ sh\text{-}2}\ ♂ \quad \ldots\ldots\ (B)$$

The results of these matings are given in Table II. There was some uncertainty in the classification of *sh-2* in the presence of *Tr*. Trembler presented no difficulty, but no double mutant, *Tr sh-2*, was identified with certainty. The classification of *sh-2* was made primarily by deafness at the age of about three weeks, but a few tremblers died before tests of deafness could be made. Some of these tremblers had shown locomotory movements resembling those of shakers and were provisionally classified as probable shakers. One of these was seen to die in a convulsion after running and circling extremely actively for some seconds. No other fatal convulsion has been observed in all the tremblers reared in the laboratory. These doubtful animals are classed as shakers in the table, but are marked with a query.

From the data in Table II it is at once apparent that position (a) is ruled out

TABLE I. Simultaneous backcross segregation of trembler and rex, in females

Parents	Progeny					Recombination
	Tr Re	*Tr* +	+ *Re*	+ +	Total	%
$\frac{Tr\ +}{+\ Re}\ ♀ \times \frac{+\ +}{+\ +}\ ♂$	11	52	54	22	139	23.7
$\frac{Tr\ Re}{+\ +}\ ♀ \times \frac{+\ +}{+\ +}\ ♂$	38	8	18	55	119	21.8
Combined						22.9

*Agricultural Research Council, Scientific Staff and holder of studentship from Commonwealth Scientific and Industrial Research Organization, respectively.

because *Tr* shows very little recombination with *sh-2*, only two certain and five doubtful recombinants having been observed in a total of 132 mice. Trembler must therefore lie close to shaker. The recombination frequency between *Tr* and *sh-2* is probably best estimated from the 69 non-trembler progeny among which were the two certain recombinants. This gives an estimate of 2.9 percent. If all the progeny and the doubtful recombinants are included the estimate is 5.3 percent.

To decide which side of *sh-2* trembler lies one considers the recombinants between *Tr* and *sh-2* (i.e. *Tr sh-2* or + + progeny) and asks which order would give the observed phenotype of these with respect to *wa-2* or *Re* by single and not double crossing over. All seven relevant recombinants agree in showing this order to be *Re - Tr - sh-2 - wa-2*, because *wa-2* goes with *sh-2* and *Re* goes with *Tr*. Since the number of relevant recombinants is so small the probability of this being an erroneous conclusion must be assessed. Suppose that the order were really *Re - sh-2 - Tr - wa-2*, and consider only the two certain recombinants between *Tr* and *sh-2*. The one in the matings involving *wa-2* would then be a double recombinant, crossing over having also taken place between *Tr* and *wa-2*. The probability of the latter event is the probability of recombination between *Tr* and *wa-2*, i.e. the recombination frequency if chiasma interference is neglected; estimated from these matings alone this is about 0.27. Similarly the one certain recombinant between *Tr* and *sh-2* in the matings involved *Re* would be a double recombinant, crossing over having also taken place between *Re* and *sh-2*; the probability of this, estimated in the same way is about 0.23. Thus the joint probability of both being double recombinants is about 0.062. This is already a small probability, and chiasma interference will reduce it still further. The alternative order which would make these double recombinants must therefore be rejected, and the order *Re - Tr - sh-2 - wa-2*, which is supported also by all the five doubtful recombinants between *Tr* and *sh-2*, must be taken as proved.

The location of trembler in the middle of the linkage group means that trembler is of little use as an additional marker in linkage studies; this will be considered fortunate by mouse geneticists, since trembler is not an easy gene to work with.

In conclusion, attention may be drawn to the very close proximity, in terms of recombination, of the three genes *Tr*, *sh-2* and *vt*, which suggests that this region of the chromosome may be one of low chiasma frequency. But our knowledge of the genetic map of the mouse is probably not yet extensive enough to justify speculation as to whether arrangements of this sort are purely fortuitous or are due to chiasma localization.

TABLE II. Backcross matings involving *Tr* with *sh-2* and *wa-2* (A), and with *sh-2* and *Re* (B). 'Z' in the progeny means *wa-2* in A-matings, and *Re* in B-matings

Mating Type	Progeny: *Tr* *sh-2* Z	*Tr* +	+ *sh-2* Z	+ +	*Tr* *sh-2* Z	*Tr* +	+ *sh-2* Z	+ +	Total
A	4?	0	2	37	18	11	0	1	75
B	1?	0	16	3	8	28	0	1	57

Mating type A = $\frac{Tr\ +\ +}{+\ sh\text{-}2\ wa\text{-}2}$ ♀ × $\frac{+\ sh\text{-}2\ wa\text{-}2}{+\ sh\text{-}2\ wa\text{-}2}$ ♂

Mating type B = $\frac{Re\ Tr\ +}{+\ +\ sh\text{-}2}$ ♀ × $\frac{+\ +\ sh\text{-}2}{+\ +\ sh\text{-}2}$ ♂

Summary

Trembler is located in linkage group VII of the mouse, between rex and shaker-2. The data show 23 ± 2.6 percent recombination between *Tr* and *Re* and about 3 percent between *Tr* and *sh-2*, both in females. The order of the genes in linkage group VII and the approximate recombination percentages in females are therefore as follows:

$$Re \xrightarrow{23} Tr \xrightarrow{3} \left\{\begin{matrix} sh\text{-}2 \\ vt \end{matrix}\right\} \xrightarrow{25} wa\text{-}2$$

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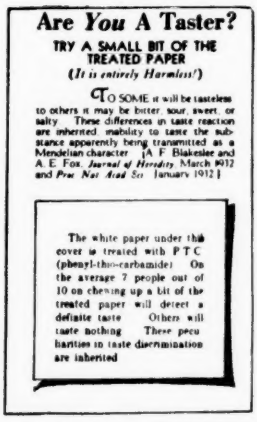
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